

# Imaging and Resection of Glioblastoma

in light of molecular markers



Fatih Incekara



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# **Imaging and Resection of Glioblastoma**

in light of molecular markers

**Beeldvorming en Resectie van Glioblastoom**  
in het licht van moleculaire markers

## **Thesis**

to obtain the degree of Doctor from the  
Erasmus University Rotterdam  
by command of the Rector Magnificus  
Prof.dr. F.A. van der Duijn Schouten

and in accordance with the decision of the Doctorate Board.

The public defense shall be held on  
Wednesday 10<sup>th</sup> of March 2021  
at 13.00 hours

by

**Fatih Incekara**  
born in Rotterdam

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**Copromotor**

Dr. A.J.P.E. Vincent

to Sara  
to Merve  
to my parents

**dare to know**

Immanuel Kant

**know thyself**

Socrates

**he who knows himself knows his Lord**

Prophet Mohammed

peace be upon him

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1. Intraoperative Ultrasound Guidance and Extent of Resection in Glioblastoma Surgery: a Randomised, Controlled Trial  
**Fatih Incekara**, Marion Smits, Linda Dirven, Eelke Bos, Rutger Balvers, Iain Haitsma, Joost Schouten, Arnaud J.P.E. Vincent.  
*Submitted*
2. The association between the extent of glioblastoma resection and survival in light of MGMT promoter methylation in 326 patients with newly diagnosed IDH wildtype glioblastoma.  
**Fatih Incekara**, Marion Smits, Sebastian R. van der Voort, Hendrik Jan Dubbink, Peggy N. Atmodimedjo, Johan M. Kros, Arnaud J.P.E. Vincent, Martin van den Bent.  
*Front. Oncol. 2020 Jul 10;10:1087.*
3. Topographical Mapping of 436 Newly Diagnosed IDH Wildtype Glioblastoma with vs. without MGMT Promoter Methylation  
**Fatih Incekara\***, Sebastian van der Voort\*, Hendrikus J. Dubbink, Peggy N. Atmodimedjo, Rishi Nandoe Tewarie, Geert Lycklama, Arnaud J.P.E. Vincent, Johan M. Kros, Stefan Klein, Martin van den Bent, Marion Smits. *Front. Oncol. 2020 May 12; 10:596.*
4. Predicting the 1p/19q co-deletion status of presumed low grade glioma with an externally validated machine learning algorithm  
Sebastian van der Voort\*, **Fatih Incekara\***, Maarten M.J. Wijnenga, Georgios Kapas, Mayke Gardeniers, Joost W. Schouten, Martijn P.A. Starmans, Rishi Nandoe Tewarie, Geert J. Lycklama, Pim J. French, Hendrikus J. Dubbink, Martin van den Bent, Arnaud J.P.E. Vincent, Wiro J. Niessen, Stefan Klein, Marion Smits.  
*Clin Cancer Res. 2019 Dec 15;25(24):7455-7462.*
5. Association between supratotal glioblastoma resection and patient survival: a systematic review and meta-analysis  
**Fatih Incekara**, Stephan Koene, Arnaud J.P.E. Vincent, Martin van den Bent, Marion Smits.  
*World Neurosurg. 2019 Jul;127:617-624.*
6. Letter to the Editor: Supratotal resection of glioblastoma  
**Fatih Incekara**, Marion Smits, Arnaud J.P.E. Vincent,  
*J Neurosurg. 2019 Aug 16:1-2.*

7. Clinical feasibility of a wearable mixed reality device in neurosurgery  
**Fatih Incekara**, Marion Smits, Clemens Dirven, Arnaud J.P.E. Vincent.  
*World Neurosurg.* 2018 Oct;118:e422-e427.
  
8. Changes in language white matter tract microarchitecture are associated with cognitive deficits in patients with presumed low grade glioma.  
**Fatih Incekara**, Djaina Satoer, Evy Visch-Brink, Arnaud J.P.E. Vincent.  
*J Neurosurg.* 2018 Jun 8:1-9.
  
9. WHO 2016 subtyping and automated segmentation of glioma using multi-task deep learning.  
 Sebastian R. van der Voort\*, **Fatih Incekara\***, Maarten MJ Wijnenga , Georgios Kapsas, Renske Gahrman , Joost W Schouten , Rishi Nandoe Tewarie, Geert J Lycklama, Philip C De Witt Hamer, Roelant S Eijgelaar, Pim J French, Hendrikus J Dubbink, Arnaud JPE Vincent, Wiro J Niessen, Martin J van der Bent, Marion Smits\*, and Stefan Klein\*.  
 Submitted
  
10. Development and external validation of a clinical prediction model for survival in glioblastoma patients.  
 Hendrik-Jan Mijderwijk, Daan Nieboer, **Fatih Incekara**, Berger K, Daniel Hänggi, Michael S Sabel, Jörg Felsberg, Guido Reifenberger, Martin J van den Bent, Marion Smits, Marie-Therese Forster, Marcel A Kamp.  
 Submitted
  
11. The Value of Pre- and Intraoperative Adjuncts on the Extent of Resection of Hemispheric Low-Grade Gliomas: A Retrospective Analysis.  
**Fatih Incekara**, Olutayo Olubiyi, Aysegul Ozdemir, Tomas Lee, Laura Rigolo, Alexandra Golby.  
*J Neurol Surg A Cent Eur Neurosurg.* 2016 Mar;77(2):79-87.
  
12. Intraoperative Magnetic Resonance Imaging in Intracranial Glioma Resection: A Single-Center, Retrospective Blinded Volumetric Study.  
 Olutayo Olubiyi, Aysegul Ozdemir, **Fatih Incekara**, Yanmei Tie, Parviz Dolati, Lianghe Hsu, Sandro Santagata, Zhenrui Chen, Laura Rigolo, Alexandra Golby.  
*World Neurosurg.* 2015 Aug;84(2):528-36.

\* contributed equally





# Chapter 1

## Introduction



## Glioma epidemiology & clinical presentation

Gliomas are brain tumors that arise from glial cells and are molecularly classified following the updated World Health Organization (WHO) 2016 Classification of Tumors of the Central Nervous System. The main types of diffuse, non-circumscribed glioma are oligodendroglioma, astrocytoma and glioblastoma. It is estimated that in 2020, over 20,000 patients will be newly diagnosed with some type of glioma in the United States.<sup>(1)</sup> Glioblastoma accounts for the majority of these tumors (57.3%) and are the most aggressive type. The age-adjusted incidence rate of glioblastoma is 3.22 per 100,000 population. The median age of patients diagnosed with glioblastoma is 65 years, with highest rates between 75-84 years. Glioblastoma is 1.58 times more common in men than in women. The etiology of glioblastoma is unknown.

The clinical presentation of brain tumors depends on tumor localization and growth rate. Diffuse astrocytoma and oligodendroglioma (low grade glioma) tend to grow more slowly than glioblastoma; they present less commonly with focal neurological deficits and more often with seizures. Glioblastoma patients present in general more often with sub-acute symptoms that progress over days to weeks, which include persistent headache, fatigue, and focal neurological symptoms, such as memory loss, motor, speech or visual deficits, cognitive and personality changes.<sup>(2,3)</sup> Seizures are less common in glioblastoma than in low grade gliomas. Magnetic resonance imaging (MRI) is needed for the radiological diagnosis of a cerebral mass lesion.

Patients with a glioma as seen on MRI are referred for surgery on a short term with the aim to undergo maximal safe tumor resection to reduce symptoms, increase survival and ultimately to obtain definitive histopathological and molecular diagnosis. If resection is deemed not feasible, a biopsy is required for tissue diagnosis. After surgery, patients are treated with a radiotherapy and/or chemotherapy scheme, which depends on factors such as age, neurological status, extent of tumor resection and molecular classification of the tumor.

## Molecular classification

The WHO 2016 classification is predominantly based on molecular characteristics, in particular mutations in the gene encoding for isocitrate dehydrogenase (IDH) 1 and 2 and 1p/19q codeletion.<sup>(4,5)</sup> Oligodendroglioma are now defined as diffuse glioma with 1p/19q codeletion and IDH mutation; astrocytoma are classified according to their IDH mutational status as either IDH mutated (mt) or wildtype (wt). The 2016 WHO currently distinguishes between glioblastoma IDHwt and IDHmt.<sup>(4,6)</sup>

More recently, low grade astrocytoma IDHwt with certain molecular characteristics (combined gain of chromosome 7 and loss of chromosome 10 and/or EGFR amplification and/or TERT promoter mutations) are called astrocytoma with molecular features of

glioblastoma. Another more recent change is the renaming of glioblastoma IDHmt as grade IV astrocytoma IDHmt.<sup>(7,8)</sup>

IDH mutations are early mutations affecting codon 132 in 90 percent of all IDH mutations in diffuse glioma. The mutation leads to changes in the enzyme and consequently in increased levels of 2-hydroxyglutamate and decreased levels of  $\alpha$ -ketoglutarate and NADPH.<sup>(9)</sup> Due to these alterations, and due to the MGMT promoter methylating effect of IDH mutation, these tumors become more sensitive for alkylating chemotherapy and radiotherapy.<sup>(6,10,11)</sup> In tumors that accumulate IDH mutation, a combined deletion of the short arm of chromosome 1 and the long arm of chromosome 19 may occur as a result of balanced translocation (oligodendroglioma).<sup>(12,13)</sup> Next to IDH mutation, 1p19q codeletion is also associated with increased sensitivity for alkylating chemotherapy.<sup>(14,15)</sup> In glioblastoma, another alteration that is associated with improved prognosis is methylation of the promoter region of the gene O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT).<sup>(16-18)</sup> MGMT is a DNA repair enzyme, which is expressed by the MGMT gene located on chromosome 10q26. Promoter methylation of this gene reduces MGMT protein expression and consequently decreases DNA repair and increases alkylating chemotherapy induced tumor death. Therefore, patients with MGMT methylated glioblastoma are more sensitive to temozolomide than those without MGMT methylated glioblastoma and thus have a better prognosis. MGMT promoter methylation is present in approximately 35-50% of patients with newly diagnosed glioblastoma.<sup>(19)</sup> IDH mutation, 1p19q codeletion and MGMT promoter methylation are all associated with more favorable prognosis in patients with glioma.<sup>(13,15,20-23)</sup>

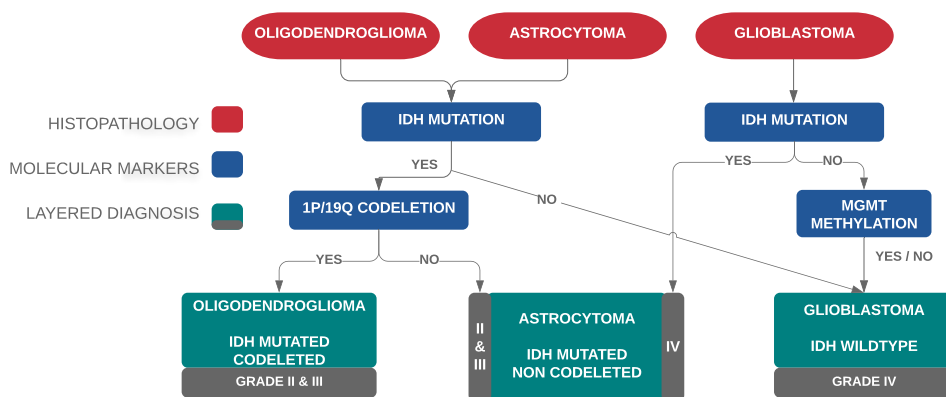
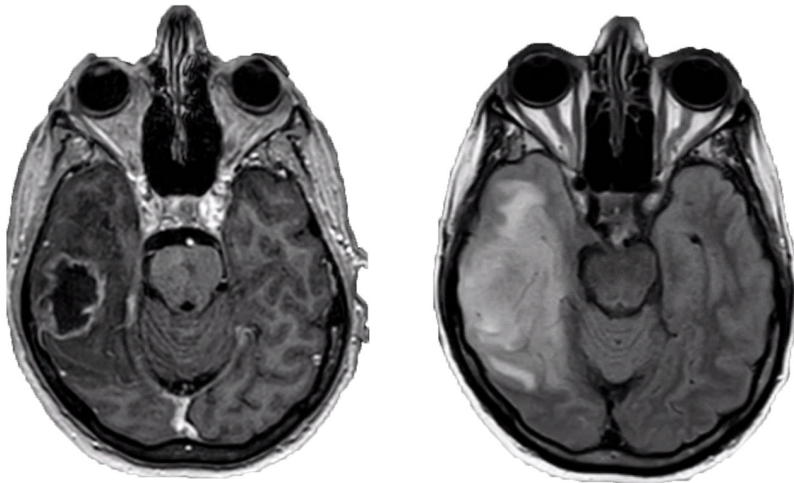


Figure 1. WHO 2016 molecular classification of glioma with cIMPACT-NOW update (brief overview).

## Imaging and radiogenomics

Diffuse oligodendroglioma and astrocytoma are hypointense on T1-weighted MRI scans and hyperintense on T2-weighted MRI scans. Radiogenomics research has the main goal to correlate anatomical and physiological MRI features with molecular subtypes, increasingly with an artificial intelligence approach.<sup>(24-31)</sup> Studies have indicated that oligodendroglioma with 1p/19q codeletion and IDH mutation are typically located in the frontal lobes with calcification, cortical-subcortical involvement, a heterogeneous appearance on T2-weighted MRI scans with indistinct borders and minimal or patchy contrast enhancement on contrast-enhanced T1-weighted MRI scans. In contrast, astrocytomas with IDH mutation and without 1p/19q codeletion are more often located in the temporal lobe or insular regions. They are homogenous on T2-weighted MRI scans with distinct borders, and they lack calcifications, cortex involvement or contrast-enhancement on contrast-enhanced T1-weighted MRI scans.

Glioblastoma typically appear as a contrast enhancing lesion with central necrosis on post-contrast T1-weighted MRI scans.<sup>(29)</sup> Glioblastoma infiltrate far beyond the margins of contrast enhancement and together with edema, this infiltration appears as a non-contrast enhanced, hyperintense area on a T2-weighted or T2-FLAIR MRI scan.<sup>(32)</sup> Advanced and physiological MRI (diffusion weighted imaging and PET-MRI) is shown to be useful to detect glioma infiltration more accurately.<sup>(33)</sup> There are currently no reliable MRI features that can distinguish MGMT promotor methylated glioblastoma from unmethylated tumors.



**Figure 2.** Glioblastoma in the right temporal lobe as seen on post-contrast T1-weighted MRI scan (left) and FLAIR-weighted MRI scan (right).

## Extent of resection

In glioblastoma, complete resection of contrast-enhancing tumor on post-contrast T1-weighted MRI has consistently been associated with longer overall survival.<sup>(34-39)</sup> A systematic review and meta-analysis of 37 articles with over 41.000 glioblastoma patients showed that complete tumor resection decreased the risk of one and two year mortality, when compared to subtotal resection.<sup>(34)</sup> In addition, more recent studies have shown that resection beyond the borders of contrast enhancement is associated with improved overall survival in patients with glioblastoma.<sup>(35, 36, 40-42)</sup> However, complete tumor resection and maximizing resection beyond the borders of contrast enhancement should not be achieved at all cost. Reports on the safety of supratotal resection (beyond the contrast enhancing borders) are still limited in numbers and therefore, needs further investigation.<sup>(42)</sup> Intraoperative imaging technologies can be used to achieve safe and maximal tumor resection during glioblastoma surgery.<sup>(43-45)</sup> Intraoperative, real time imaging is needed, since neuronavigation systems are typically based on preoperative MRI scans and due to brain shift, their accuracy in representing the actual situation during surgery decreases. Two randomized controlled trials have shown that 5-aminolevulinic acid and intraoperative MRI guided surgery improves the extent of glioblastoma resection.<sup>(43-45)</sup> However, an intraoperative MRI system is expensive and prolongs surgery time with approximately one hour.<sup>(45)</sup> Alternative time- and cost- effective imaging technologies may be useful, such as intraoperative Raman spectroscopy or intraoperative ultrasound guidance.<sup>(43,46,47)</sup> However, no randomized controlled trial has assessed their value to improve the extent of glioblastoma resection and overall outcome.<sup>(43)</sup>

Despite improved surgical and imaging techniques, maximization of the extent of resection and the addition of temozolomide to radiotherapy over the past few decades, glioblastoma patients still have a poor prognosis of 15 months (6.8% five-year overall survival rate).<sup>(1,48-50)</sup> Patients eventually show disease progression and die due to mass effect or extensive brainstem infiltration.<sup>(51,52)</sup> There is currently no cure for glioblastoma.

## Aims and outline of thesis

In this thesis, we assessed the value of glioblastoma imaging and resection in light of molecular markers.

In **Part I** of this thesis, our aim was to predict molecular markers of glioma on preoperative MRI scans. In **Chapter 2** we voxel-wise analyze whether there is a difference in anatomical localization between IDH wildtype glioblastoma with vs. without MGMT promoter methylation. In **Chapter 3 and 4** we predict molecular subtypes of glioma (i.e. 1p/19q codeletion, IDH mutation, MGMT promoter methylation) based on MRI scans using

machine and deep learning algorithms. In **Chapter 5** we evaluate cognitive functions of patients with glioma prior to surgery using white matter fiber tracking.

In **Part II** of this thesis, we assessed the value of image guided glioblastoma resection. In **Chapter 6** we present the results of the ultrasound trial, which is a randomized controlled trial that assesses the value of intraoperative ultrasound guided surgery on the extent of glioblastoma resection. The question whether intraoperative ultrasound guided surgery enables complete tumor resection more often than standard surgery will be answered. In **Chapter 7** we evaluate the clinical feasibility of a wearable mixed reality device for planning glioblastoma surgery, presenting the first proof of concept.

**Part III** of this thesis consists of studies providing a postoperative evaluation of glioblastoma resection. In **Chapter 8** we assess the association between the resection of contrast enhancing and non-contrast enhancing parts of the tumor and survival in light of MGMT promoter methylation in a cohort of patients with newly diagnosed IDH wildtype glioblastoma. We answer the question whether complete resection is associated with improved survival in patients with molecularly defined glioblastoma. In relation to this, in **Chapter 9** we perform an international, multicenter, observational study, including over one thousand patients with a newly diagnosed IDH-wildtype glioblastoma, in which we develop and externally validate a survival prediction model. In **Chapters 10.1 and 10.2** we systematically review and assess the value of supratotal resection on patient survival, we present a meta-analysis and an editorial letter on this concept.

Finally, in **Chapter 11**, I provide a summary and an overall conclusion of this thesis.

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# PART I

## Preoperative imaging





# Chapter 2

## Topographical Mapping of 436 Newly Diagnosed IDH Wildtype Glioblastoma with vs. without MGMT Promoter Methylation

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## **Abstract**

### **Background**

O<sup>6</sup>-methylguanine-methyltransferase (MGMT) promoter methylation and isocitrate dehydrogenase (IDH) mutation status are important prognostic factors for patients with glioblastoma. There are conflicting reports about a differential topographical distribution of glioblastoma with vs. without MGMT promoter methylation, possibly caused by molecular heterogeneity in glioblastoma populations. We initiated this study to re-evaluate the topographical distribution of glioblastoma with vs. without MGMT promoter methylation in light of the updated WHO 2016 classification.

### **Methods**

Pre-operative T2-weighted/FLAIR and post-contrast T1-weighted MRI scans of patients aged 18 year or older with IDH wildtype glioblastoma were collected. Tumors were semi-automatically segmented and the topographical distribution between glioblastoma with vs. without MGMT promoter methylation was visualized using frequency heatmaps. Then voxel-wise differences were analyzed using permutation testing with Threshold Free Cluster Enhancement.

### **Results**

Four hundred thirty-six IDH wildtype glioblastoma patients were included; 211 with and 225 without MGMT promoter methylation. Visual examination suggested that when compared with MGMT unmethylated glioblastoma, MGMT methylated glioblastoma were more frequently located near bifrontal and left occipital periventricular area and less frequently near the right occipital periventricular area. Statistical analyses, however, showed no significant difference in topographical distribution between MGMT methylated vs. MGMT unmethylated glioblastoma.

### **Conclusion**

This study re-evaluated the topographical distribution of MGMT promoter methylation in 436 newly diagnosed IDH wildtype glioblastoma, which is the largest homogenous IDH wildtype glioblastoma population to date. There was no statistically significant difference in anatomical localization between MGMT methylated vs. unmethylated IDH wildtype glioblastoma.



## Introduction

Patients with glioblastoma have a poor prognosis with a median overall survival of 15 months, despite standard of care consisting of safe and maximal surgical resection followed by chemo- and/or radiotherapy.<sup>(1)</sup> This prognosis varies based on factors such as age, Karnofsky Performance Status, extent of resection and molecular markers, in particular isocitrate dehydrogenase (IDH) mutation and O<sup>6</sup>-methylguanine-methyltransferase (MGMT) promoter methylation status.<sup>(2)</sup>

MGMT is a DNA repair enzyme, which is expressed by the MGMT gene located on chromosome 10q26. Promoter methylation of this gene reduces MGMT protein expression and consequently decreases DNA repair and increases alkylating chemotherapy induced tumor death. Therefore, patients with MGMT methylated glioblastoma are more sensitive to neo-adjuvant temozolomide than those without MGMT methylated glioblastoma. MGMT is methylated in approximately 50% of patients with newly diagnosed glioblastoma.<sup>(3)</sup>

There are conflicting results in the published literature on a possible differential topographical distribution of glioblastoma with vs. without MGMT promoter methylation.<sup>(4)</sup> Ellingson et al. suggested that when compared with those without MGMT promoter methylation, glioblastoma with methylation are more frequently located in the left temporal lobe and less frequently in the right temporal lobe.<sup>(5)</sup> However, other studies found the reverse lateralization pattern<sup>(6)</sup> or did not find any lateralization at all.<sup>(7-9)</sup> These conflicting results could be ascribed to heterogeneity of molecular subtypes of glioblastoma in the studied populations, for instance when IDH wildtype glioblastoma are mixed with the genetically and prognostically distinct IDH mutated glioblastoma, or to variation in statistical methods that were used across studies. Therefore, the question whether glioblastoma with vs. without MGMT promoter methylation have a different anatomical localization remains unanswered. In light of the updated WHO 2016 classification<sup>(10)</sup>, a molecularly homogenous glioblastoma population must be used to re-evaluate the topographical distribution of MGMT methylated vs. unmethylated glioblastoma.

Therefore, we have initiated this study to re-evaluate the topographical distribution of glioblastoma with and vs. without MGMT promoter methylation in the largest homogenous IDH wildtype glioblastoma population to date.

## METHODS

### Patient inclusion

All consecutive patients aged 18 years or older newly diagnosed with a contrast-enhancing and histopathologically confirmed glioblastoma IDH wildtype who underwent tumor resection or biopsy between January 2011 and May 2018 at the Erasmus MC, University Medical Center Rotterdam or Haaglanden MC were retrospectively included in

this study. Patients were eligible if pre-operative T2-weighted/fluid-attenuated inversion recovery (FLAIR) and post-contrast T1-weighted MRI scans as well as molecular data on IDH mutation and MGMT methylation status were available. Recurrent glioblastoma or confirmed IDH mutated glioblastoma were excluded. The study design was approved by the Medical Ethical Committee of Erasmus MC and Haaglanden MC. The study was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

### **Image acquisition, tumor segmentation and registration**

From clinical pre-operative MRI scans, which were obtained according to clinical brain tumor protocols on either a 1.5T or 3.0T scanner, T2-weighted/FLAIR and post-contrast T1-weighted images were collected. For glioblastoma segmentation, we first imported both the post-contrast T1-weighted and T2-weighted/FLAIR scans into BrainLab (BrainLab, Feldkirchen, Germany, version 2.1.0.15). We semi-automatically segmented all tumor-related contrast-enhancement (including the central necrotic part, if present) using the SmartBrush tool in Brainlab Elements and manually adapted the segmentation if needed. We then used the T2-weighted/FLAIR scan to semi-automatically segment all tumor-related non-enhancing hyperintense abnormalities (extra-lesional hemorrhage were excluded).

All tumor segmentations were then registered to the Montreal Neurological Institute (MNI) International Consortium for Brain Mapping 152 nonlinear atlas. The post-contrast T1-weighted scans were registered to the T1-weighted atlas and the T2-weighted/FLAIR scans to the T2-weighted atlas. Registration was done using SimpleElastix (version 72b7e81), based on a mutual information metric using an affine registration.<sup>(11)</sup> The resulting transformation parameters were used to transform the 3D segmentations to the atlas space. Registration results were visually checked to ensure that for all cases the registered masks lay entirely within the brain mask of the atlas. No adjustments were made to the initial registration settings for individual patients. We created voxel-wise frequency maps for all glioblastoma combined, and frequency difference maps of glioblastoma with versus without MGMT promoter methylation.

### **Molecular analysis**

Tumor tissue samples were obtained from patients through surgical resection or biopsy. Histopathological examination was performed by neuropathologists. DNA was extracted from microdissected FFPE tissue fragments by proteinase K digestion for 16 h at 56 C in the presence of 5 % Chelex 100 resin and used after inactivation of proteinase K and removal of cell debris and the Chelex resin. IDH mutational analysis was assessed with Sanger sequencing of PCR-amplified fragments from IDH1 and IDH2 mutational hotspots, essentially as previously described.<sup>(12)</sup> M13-tailed primers for PCR amplification of IDH1 codon 132 were forward 5'-TGTAAC ACGACGGCCAGTCTCCTGATGAGAAGAGGGTTG-3' and reverse 5'-CAGGAAACAGCTATGACCCATT CTCTGGTTTTCGCATGCAAAATCACATTATTGCC-3'. After

initial denaturation at 95 C for 3 min, 35 cycles of 95 C for 30 s, 60 C for 45 s, and 72 C for 45 s were performed, followed by 10 min at 72 C. Subsequent sequence analyses of the PCR products was carried out with M13 forward and reverse primers on an 3730 XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Targeted NGS was performed by semiconductor sequencing with the Ion Torrent platform using supplier's materials and protocols (Thermo Fisher Scientific) with a dedicated panel for detection of glioma-specific aberrations, including IDH1 and IDH2 hot spot mutations essentially as previously described.<sup>(13)</sup> Library and template preparations were performed consecutively with the AmpliSeq Library Kit 2.0-384 LV and the Ion 510/520/530 Chef kit. Sequencing was performed on a 530 or 540 chip with the Ion S5 XL system. Data was analyzed with the Torrent variant caller (Thermo Fisher Scientific) and variants were annotated in a local Galaxy pipeline using ANNOVAR. Details of the glioma panel are described in the supplementary data of Dubbink et al.<sup>(13)</sup>

MGMT promoter methylation status was assessed by methylation-specific PCR essentially as described by Esteller et al.<sup>(14)</sup> Bisulfite conversion and subsequent purification is performed with the EZ DNA Methylation-Gold Kit (Zymo Research) according to the supplier's protocol. Methylation-specific PCR was performed with primers specific for either methylated or the modified unmethylated DNA. Converted primer sequences for unmethylated DNA were forward 5'-TTTGTGTTTGTATGTTTGTAGGTTTTGT-3' and reverse 5'-AACTCCACACTCTTCCAAAAACAAAACA-3'; and for the methylated reaction, forward 5'-TTTCGACGTTCTAGGTTTTCGC-3' and reverse 5'-GCACTCTTCCGAAAACGAAACG-3'. PCR was performed after initial denaturation at 95 C for 5 min by 35 cycles of 92 C for 45 s, 59 C for 65 s, and 72 C for 45 s, followed by 7 min at 72 C. Five microliters of each 15 µl methylation-specific PCR product was loaded onto a 1.5 % agarose gel stained with GelRed (Biotium) and examined under ultraviolet illumination. SW48 cell line DNA and tonsil DNA was used as a positive control for methylated and unmethylated alleles of MGMT, respectively. Controls without DNA were used for each set of methylation-specific PCR assays.

### Statistical analysis

We first tested the differences between pre-operative enhancing and non-enhancing tumor volumes as well as their ratio with the Kruskal-Wallis test. We mapped the anatomical localization of all MGMT methylated and unmethylated glioblastoma by iterating over all voxels in the MNI atlas and counting the number of tumor frequencies for each group in each voxel. To test for differences in spatial distribution between glioblastoma with vs. without MGMT promoter methylation, we assessed the cluster-wise significance at the voxel-level between distributions, using permutation testing with Threshold Free Cluster Enhancement<sup>(15)</sup> in the software package "FSL Randomize" (version 5.0.9, using 10,000 permutations).<sup>(16)</sup> This approach corrects p-values for the family-wise error in testing multiple voxels, considering a corrected p-value of <0.05 as statistically significant.

Results

In total, 769 patients with newly diagnosed, contrast enhancing glioblastoma were screened, of which we excluded 333 patients: 22 were excluded due to IDH mutation and 311 were excluded due to insufficient or missing molecular data on IDH mutation or MGMT methylation status. Final analysis included 436 patients with IDH wildtype glioblastoma (see flowchart, Supplementary Material); 211 with and 225 without MGMT promoter methylation. 340 patients had undergone a surgical tumor resection and 96 a diagnostic biopsy. In all patients pre-operative post-contrast T1-weighted MRI scans were available; in 90 patients T2-weighted FLAIR scans and in 346 patients T2-weighted scans were available. When compared with MGMT unmethylated glioblastoma, MGMT methylated glioblastoma had a significantly higher ratio of non-enhancing versus contrast-enhancing volume (2.09 (inter quartile range 2.6) and 2.5 (inter quartile range 3.3),  $p=0.045$ , respectively). Patient and tumor characteristics are further presented in Table 1.

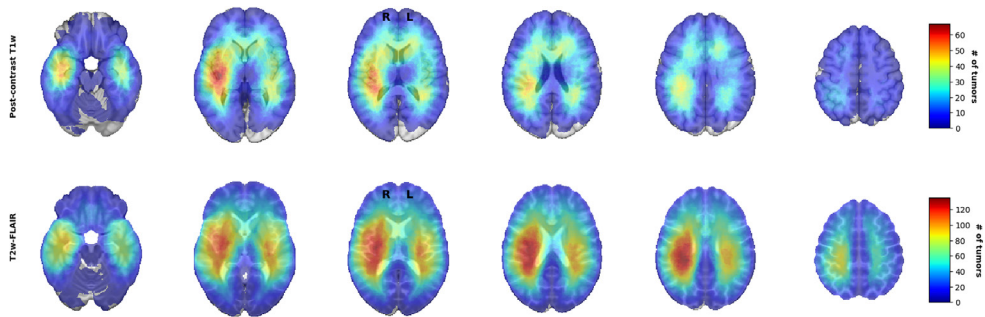
Table 1. Patient and tumor characteristics.

Characteristics	n	%	
<i>all patients</i>	436	100	
<b>Sex</b>			
Male	276	63.3	
Female	160	36.7	
<b>Age</b>	x	x	
≤ 65	227	52.1	
> 65	209	47.9	
Mean, years (SD)	61.5 (16.2)		
<b>Karnofsky Performance Status</b>			
≤ 70	142	32.6	
> 70	294	67.4	
Mean (SD)	80 (12.5)		
<b>Pre-operative MRI scans</b>			
T1 post-contrast	436	100	
T2-weighted	346	79.4	
T2-weighted FLAIR	90	20.6	
<b>Neurosurgical procedure</b>			
Resection	340	78.0	
Biopsy	96	22.0	
	<b>MGMT promotor</b>		
<b>Pre-operative volume, median cm<sup>3</sup> (IQR)</b>	<b>Methylated</b>	<b>Unmethylated</b>	p- value
	211 (48.4%)	225(51.6%)	
Contrast-enhancing	30.1 (39.5)	35 (45.8)	
Non-enhancing	75.5 (105.0)	65.5 (84.2)	
Non-enhancing/contrast-enhancing Ratio	2.5 (3.3)	2.09 (2.6)	

SD standard deviation, IQR Inter Quartile Range, CE contrast enhancement, FLAIR Fluid-attenuated inversion recovery.

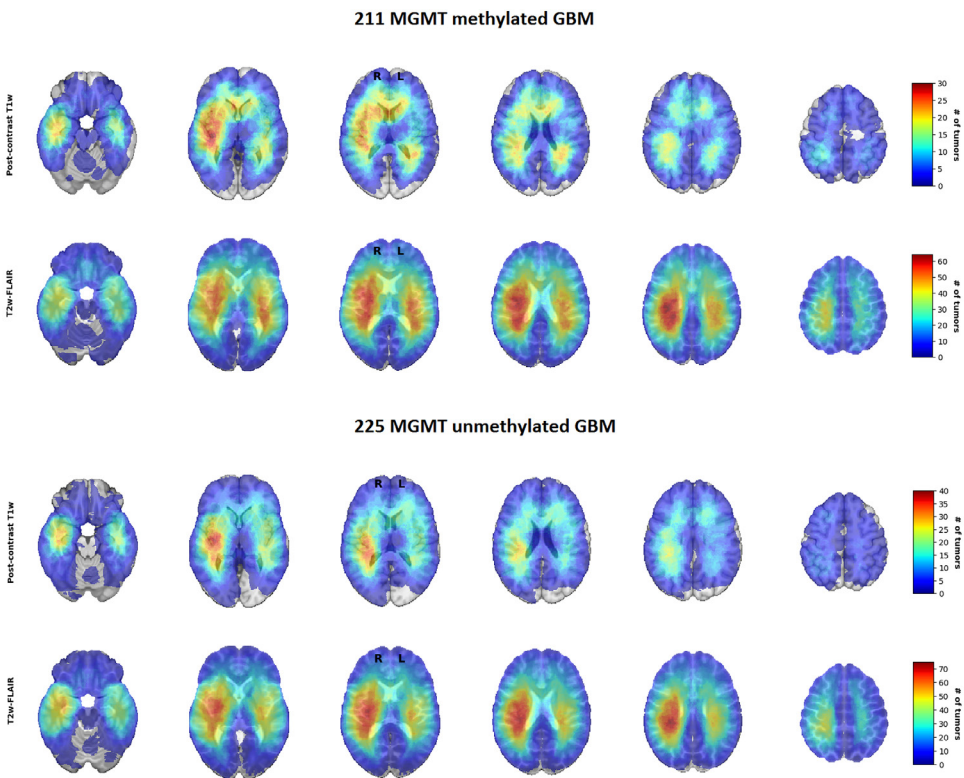
### Topographical mapping of 436 IDH wildtype glioblastoma

For visual inspection, heatmaps based on post-contrast T1-weighted and T2-weighted/FLAIR segmentations were created for all 436 patients combined (Figures 1 and 2), as well as frequency difference maps between MGMT methylated vs. unmethylated glioblastoma (Figure 3). Visual inspection of maps in Figure 1 suggests that glioblastoma were most frequently located in the right temporal, insular and parietal area, and near the periventricular area both frontally and occipitally. Visual inspection of Figures 2 and 3 indicates that when compared with MGMT unmethylated glioblastoma, methylated glioblastoma were more frequently located near bifrontal and left occipital periventricular area (up to 6.5% frequency difference) and less frequently near the right occipital periventricular area (up to 9.1% frequency difference).

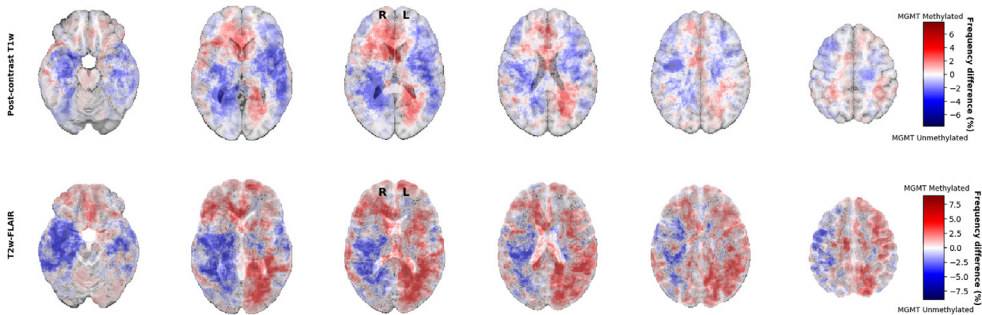


**Figure 1.** Heatmaps of all 436 IDH wildtype GBM.

To test whether this difference was statistically significant, voxel-wise analyses of both the post-contrast T1-weighted and T2-weighted/FLAIR segmentation heatmaps were performed. Although statistical analysis of the post-contrast T1-weighted scans marked a region near the right occipital periventricular area as a potentially discriminating area between MGMT methylated vs. unmethylated glioblastoma, this difference was not statistically significant (Figure 4, together with corresponding p-values). This figure in fact shows that not any statistically significantly discriminating brain area between MGMT methylated and unmethylated glioblastoma could be found. Scroll-through video clips for visual inspection of all topographic maps are publicly available as Supplementary Material.

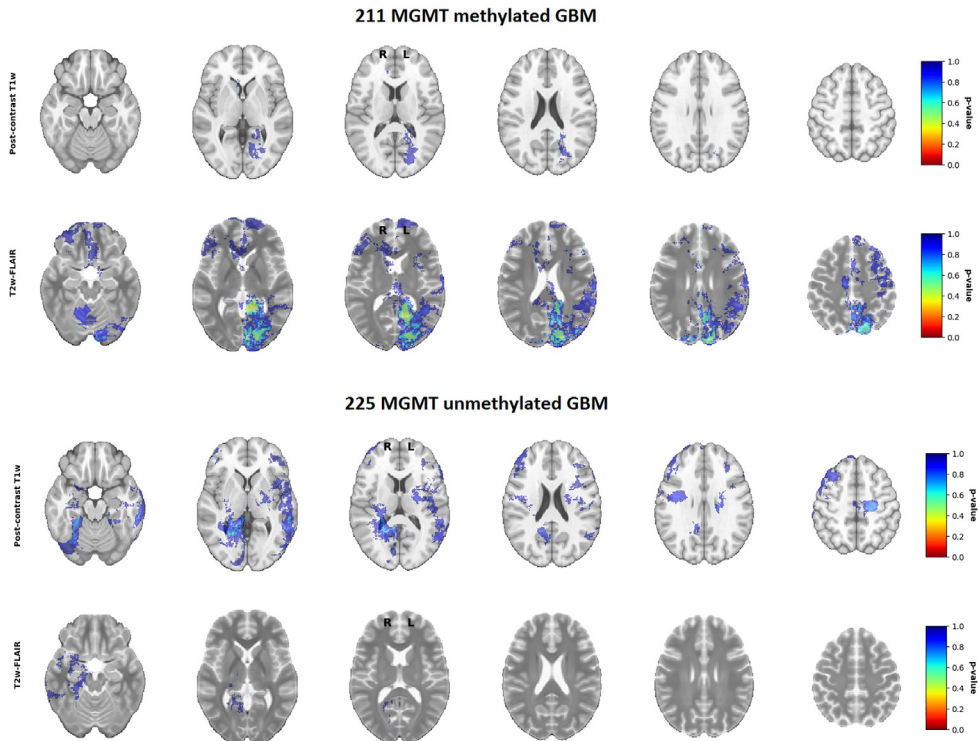


**Figure 2.** Heatmaps of MGMT methylated (N=211) and unmethylated (N=225) GBM.



**Figure 3.** Frequency difference maps between MGMT methylated (N=211) and unmethylated (N=225) GBM.





**Figure 4.** P-value maps of MGMT methylated (N=211) and unmethylated (N=225) GBM.

## Discussion

This study voxel-wise analyzed post-contrast T1-weighted and T2-weighted/FLAIR heatmaps and showed that there was no statistically significant difference in anatomical localization between MGMT methylated vs. unmethylated IDH wildtype glioblastoma.

The primary reason to initiate this study was to re-evaluate the anatomic localization of MGMT methylated vs. unmethylated glioblastoma in light of the updated WHO 2016 classification era following conflicting reports on this topic.<sup>(4)</sup> Ellingson et al. (2013) reported that glioblastoma with MGMT methylation were lateralized to the left hemisphere (temporal lobe) and that those without were lateralized to the right hemisphere (17), which was in line with their previous article (2012) and in which they included a substantial portion of their previously studied glioblastoma population.<sup>(5)</sup> However, in contrast to these findings there are also studies that found the reverse pattern of hemispheric lateralization, in which glioblastoma with MGMT methylation were located more frequently in the right hemisphere, while those without MGMT methylation lateralized to the left hemisphere.<sup>(6)</sup> Additionally, there are conflicting reports on lobar distribution, in which glioblastoma with MGMT methylation were more frequently located in the parietal

and occipital lobes, while those without were located more frequently in the temporal lobes.<sup>(8)</sup> A recent study suggested after qualitative analyses that subventricular zones were more frequently spared with MGMT methylated glioblastoma, but found no difference in hemispheric lateralization between glioblastoma with and without MGMT promoter methylation.<sup>(9)</sup> Finally, there are also studies that report no differences in localization between glioblastoma with and without MGMT methylation,<sup>(7, 18)</sup> in concordance with the findings of our study.

These conflicting results in the literature can potentially be ascribed to two methodological issues. First, inconsistencies may arise from variations in glioblastoma patient populations across studies, many of which were performed in the pre-WHO 2016 classification era when the impact of molecular subtyping of glioblastoma according to IDH mutation status was less of a consideration.<sup>(10)</sup> Ellingson et al. (2013) included a series of 507 de novo glioblastoma with mixed IDH subtypes, including 366 IDH wildtype, 34 IDH mutated glioblastoma and also 107 glioblastoma without data on IDH mutation status.<sup>(17)</sup> Moreover, the majority of the studies did not report the IDH mutation status of included glioblastoma.<sup>(5, 6, 8, 18)</sup>

Mixing molecular subtypes or not knowing IDH mutation status of glioblastoma is undesirable when assessing topographical distribution of molecular subtypes,<sup>(10)</sup> since it is now known that IDH mutated glioblastoma represent a distinct molecular subtype of glioblastoma from a distinct precursor lesion which have a predominantly frontal lobe involvement when compared with IDH wildtype glioblastoma.<sup>(19)</sup> This topographic link between IDH mutation and MGMT methylation was also suggested by Ellingson et al. (2013) by demonstrating that IDH mutated and MGMT methylated glioblastoma were indeed more frequently localized in the frontal lobe.<sup>(17)</sup> This has not only been demonstrated in glioblastoma, but also in non-contrast enhancing low grade glioma in which IDH mutated low grade glioma (both oligodendroglioma and astrocytoma) were more frequently located in the frontal lobes, while non-contrast enhancing IDH wildtype astrocytoma were more frequently located in the basal ganglia of the right hemisphere.<sup>(20)</sup> This topographical link thus suggests IDH mutation status as (confounding) factor between MGMT methylation status and localization. Therefore, studies must be conducted based on homogeneous tumor populations with respect to IDH mutational status. This hypothesis was recently supported by Roux et al, who assessed a homogenous IDH wildtype glioblastoma population (n = 392) and found no difference in localization between glioblastoma with and without MGMT methylation, in line with our study.<sup>(21)</sup>

Second, the conflicting results in the literature may arise from different statistical methods that were used across studies. Studies often investigated the anatomic localization of glioblastoma with and without MGMT promoter methylation with visual examination, qualitatively, without a statistical, voxel-wise quantitative analysis.<sup>(7-9, 18)</sup> Ellingson et al. (2013) used frequency difference maps to demonstrate that MGMT methylated glioblastoma were more frequently localized in the left temporal lobe.<sup>(17)</sup>



Using similar frequency difference maps, we also found topographical differences, which indicated that when compared with MGMT unmethylated glioblastoma, MGMT methylated glioblastoma were more frequently localized near bifrontal and right occipital periventricular area and less frequently near the right occipital periventricular area. However, we showed that these apparent differences did not survive rigorous statistical testing. Ellingson et al. report the use of 'Analysis of Differential Involvement' for their statistical analysis, which is based on the Fisher exact test.<sup>(5)</sup> We used 'FSL randomize', which is different from the Fisher exact test because it does not make any assumptions about the underlying distribution of the variables.<sup>(16)</sup> Another methodological difference can be found in the correction for multiple comparisons. Ellingson et al. used random permutations based on Bullmore et al. instead of the more recently proposed and widely accepted method of doing random permutations employed in 'FSL randomize' based on Smith et al.<sup>(15,22)</sup> Furthermore, the method by Bullmore et al. requires a user-defined threshold for clustering, which can impact the results substantially.<sup>(22)</sup> Instead, we used 'Threshold Free Cluster Enhancement', which does not require thresholding to determine the clusters, and which has been shown to have a higher sensitivity compared to other methods.<sup>(15)</sup> Our stringent methodology of rigorous statistical testing and applying new insights in glioblastoma molecular subtyping to a large studied patient population are the strengths of our study.

### Limitations

The main limitation of this study is its retrospective design, which may have introduced selection and confounding biases. Selection bias may occur when patients who receive diagnostic biopsies are excluded from analysis, since these tumors are often large, multifocal, located deep within the basal ganglia, or crossing midline. This may skew the results on tumor localization of glioblastoma, which is our main outcome. We have therefore attempted to limit this bias first by consecutive inclusion of all glioblastoma patients operated upon between 2011 and 2018 in our cohort, including diagnostic biopsies. In addition, it is known that tumor localization is associated with IDH mutation status, with IDH mutated tumors located more frequently in the frontal lobes, as mentioned earlier.<sup>(19)</sup> Since IDH mutation status is both associated with tumor localization and MGMT methylation status, it may function as a confounding factor. We therefore have also attempted to limit this potential bias by excluding all IDH mutated tumors. Another limitation is that we included patients from two medical centers from a period of over seven years. This introduced variation of MRI scan protocols such as magnet strength, voxel size and slice thickness, which consequently may have negatively influenced registration accuracy and anatomical localization. Such registration inaccuracies can however be considered minor relative to the size of the tumor and it is therefore unlikely that our results were significantly impacted by scanner variations. Additionally, tumor volume assessment on these MRI scans were performed by one observer without

confirmation of a second, independent assessor. This may have introduced some degree of information bias. We have attempted to limit this bias during volumetric assessment by blinding the assessor for patients' clinical and molecular characteristics. It is known that both the inter and intra-observer agreement for pre-operative tumor volumes in glioblastoma is relatively high.<sup>(23)</sup> Finally, it should be noted that the known intertest variability is a limitation of MGMT analyses, as assays used in other studies may produce slightly different MGMT methylation results.<sup>(24)</sup> This may partially explain the variety in the proportion of MGMT methylated tumors reported in literature.

## **Conclusion**

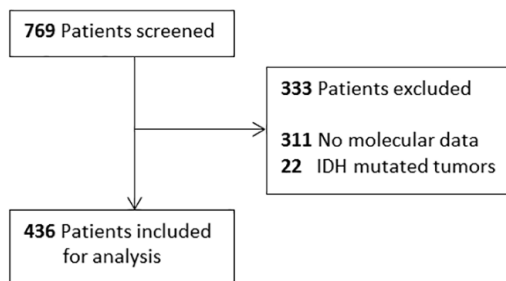
In the largest homogenous IDH wildtype glioblastoma population to date, we showed that visual appearance of differences could not be confirmed with rigorous voxel-wise statistical testing and thus that there is no statistical difference in anatomical localization between IDH wildtype glioblastoma with vs. without MGMT promoter methylation.

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## Supplementary materials



**Figure S1.** Flowchart.

The following heatmaps are online available:

- Heatmaps all GBM (T1w and T2w).gif
- Heatmaps methylated and unmethylated (T1w and T2w).gif
- Frequency difference maps (T1w and T2w).gif
- P value maps methylated and unmethylated (T1w and T2w).gif



# Chapter 3

## Predicting the 1p/19q co-deletion status of presumed low grade glioma with an externally validated machine learning algorithm

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\* contributed equally



## **Abstract**

### **Background**

Patients with 1p/19q co-deleted low-grade glioma (LGG) have longer overall survival and better treatment response than patients with 1p/19q intact tumors. Therefore, it is relevant to know the 1p/19q status. To investigate whether the 1p/19q status can be assessed prior to tumor resection, we developed a machine learning algorithm to predict the 1p/19q status of presumed LGG based on preoperative magnetic resonance imaging (MRI).

### **Methods**

Preoperative brain MRI scans from 284 patients who had undergone biopsy or resection of presumed LGG were used to train a support vector machine algorithm. The algorithm was trained based on features extracted from T1-weighted and T2-weighted MRI scans, and on patient age and sex. The performance of the algorithm compared to tissue diagnosis was assessed on an external validation dataset of MRI scans from 129 LGG patients from The Cancer Imaging Archive (TCIA). Four clinical experts also predicted the 1p/19q status of the TCIA MRI scans.

### **Results**

The algorithm achieved an area under the curve (AUC) of 0.72 in the external validation dataset. The algorithm had a higher predictive performance than the average of the neurosurgeons (AUC 0.52), but lower than that of the neuroradiologists (AUC 0.81). There was a wide variability between clinical experts (AUC 0.45-0.83).

### **Conclusion**

Our results suggest that our algorithm can non-invasively predict the 1p/19q status of presumed LGG with a performance that on average outperformed the oncological neurosurgeons. Evaluation on an independent dataset indicates that our algorithm is robust and generalizable.



## Introduction

Low grade glioma (LGG) are primary brain tumors that originate from glial cells. The World Health Organization (WHO) 2016 criteria recognize three subtypes based on molecular and histological features:<sup>(1)</sup> diffuse IDH wildtype astrocytoma (IDH wildtype, 1p/19q intact),<sup>(2)</sup> diffuse IDH mutant astrocytoma (IDH mutated, 1p/19q intact); and (3) oligodendroglioma (IDH mutated, 1p/19q co-deleted).<sup>(1,2)</sup>

Studies have shown that the distinction between these three categories is clinically relevant in terms of prognosis and management: in patients treated with optimal surgical resection followed by radiation therapy with or without chemotherapy, median survival is longest of those with oligodendroglioma.<sup>(3,4)</sup> Additionally, studies have suggested that residual tumor has a more negative impact on survival in 1p/19q intact, IDH mutated astrocytoma than on 1p/19q co-deleted, IDH mutated oligodendrogliomas.<sup>(5,6)</sup> Therefore, the ability to predict the molecular subtypes of LGG at an early stage could provide better guidance of risk-benefit assessment and clinical decision-making.

The recent shift from histopathology-based glioma classification to the molecular subtype-based WHO 2016 classification gave rise to neuro-oncological radiogenomics research in which features seen on preoperative magnetic resonance imaging (MRI) scans are used to predict the genetic mutation status of glioma.<sup>(7-9)</sup> Features such as frontal tumor localization, indistinct tumor borders, heterogeneous signal intensity on T2-weighted images, and both cortical and subcortical tumor infiltration all suggest the presence of 1p/19q co-deletion.<sup>(7)</sup>

One way of linking MRI features to 1p/19q co-deletion is through machine learning. While several studies have applied this method to datasets of patients with high grade glioma, few studies have developed radiogenomics methodology in LGG.<sup>(10-15)</sup> Of the ones that have, most have not used an independent test set and, therefore, it is difficult to estimate their actual performance in the real-world clinical setting.<sup>(10,11,13,14)</sup> Lu et al.<sup>(12)</sup> did use an independent test set, but this set contained a very limited number of LGG cases (N=12). Zhou et al.<sup>(15)</sup> used a test set consisting of IDH-mutated LGG and high-grade glioma to evaluate the 1p/19q co-deletion prediction performance. This is not an ideal test set as 1p/19q co-deletion status is not clinically relevant for high grade glioma, and there is a selection bias of IDH mutated tumors only.

The aim of this retrospective study was to develop a radiogenomics approach to predict the 1p/19q co-deletion status of presumed LGG based on pre-operative MRI features, with a machine learning algorithm that was validated on a large external dataset.

## Methods

### EMC/HMC Dataset

#### Study participants

All patients aged 18 years or older newly diagnosed with presumed LGG and who underwent tumor resection or biopsy between October 2002 and March 2017 at the Erasmus MC, University Medical Center Rotterdam (EMC) or Haaglanden Medical Center (HMC) were retrospectively included in the EMC/HMC dataset. Patients were eligible if histopathological diagnosis with molecular subclassification of the 1p/19q co-deletion status and pre-operative post-contrast T1-weighted and T2-weighted MRI scans were available. The study was approved by the Medical Ethical Committee of Erasmus MC, who waived the need for written informed consent from the patients due to the retrospective nature of this study and the (emotional) burden that would result from contacting the patients or their relatives to obtain consent. The study was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

#### Histopathological diagnosis and molecular subclassification

Tumor samples were obtained from patients who underwent surgical resection or biopsy. Histopathological examination was performed by neuropathologists and further molecular subclassification of the 1p/19q co-deletion and/or IDH mutation status was performed as part of the diagnostic routine by molecular biologists using fluorescence in situ hybridization (FISH), Loss of Heterozygosity (LOH) analysis, targeted Next-Generation Sequencing (NGS) panel using an Ion Torrent Personal Genome Machine (Life Technologies) or Ion S5XL or a Multiplex Ligation Probe Assay (MRC-Holland).<sup>(4,16-18)</sup> All tumors were subclassified based on the WHO 2016 criteria.

#### Imaging acquisition and post-processing

MRI scans were used that were acquired in the routine diagnostic process. T1-weighted and T2-weighted MRI sequences were used for the algorithm. In many, but not all, patients T2-weighted fluid attenuated inversion recovery (T2w-FLAIR) imaging was also available. As scans were acquired at a number of sites, the imaging data were heterogeneous with a wide range of acquisition settings in voxel spacing, matrix size, echo time, repetition time, number of slices, slice thickness, and field strengths on scanners from three different manufacturers (General Electric, Philips and Siemens). An overview of the scanning settings is given in the Supplementary Materials, Appendix 1.

All scans were visually inspected by M.S. and excluded if MRI artefacts were present. Presumed LGG was defined as non-enhancing tumor, as seen on the presurgical post-contrast T1-weighted MRI scan. Therefore, all post-contrast T1-weighted scans were reviewed and excluded if clear or solid enhancement was present. When available T1-weighted pre-contrast scans were inspected for hemorrhage, to prevent false positive

assessment of enhancement. Although tumors with evident contrast enhancement were excluded, minimal enhancement was tolerated. Minimal enhancement was defined as punctiform (<1mm in diameter) or poorly defined faint enhancement, similar to Pallud et al.<sup>(16)</sup>

Tumor segmentation was performed by two independent observers (F.I. and G.K.) using ITKSnap.<sup>(20)</sup> Segmentation was done on T2w-FLAIR when available (N=119), otherwise on the T2-weighted scans (N=165). Since in our institution LGG segmentations are preferably performed on T2w-FLAIR scans, we did not enforce the assessors to segment on T2-weighted scans in order to stick to the real-world clinical practice. The segmentations were then transformed to the T2-weighted scans (in the case of T2w-FLAIR segmentation) and the T1-weighted scans, using the image registration software SimpleElastix<sup>(21)</sup>. For all patients, brain masks were automatically constructed using FSL's BET tool with a fractional intensity threshold of 0.5.<sup>(22)</sup> These brain masks were subsequently used to normalize the intensity of the MRI scans. Details can be found in Appendix 2.

### TCIA Dataset

Patients from The Cancer Imaging Archive (TCIA) "LGG-1p19qDeletion" dataset were screened for eligibility based on previously described inclusion and exclusion criteria, and used as the external validation dataset.<sup>(10,23,24)</sup>

This data collection is a publicly available dataset that consists of histopathological proven LGG with co-registered T1- and T2-weighted preoperative MRI scans as well as biopsy proven 1p/19q co-deletion status. Molecular analysis of the 1p/19q co-deletion status was performed with FISH for all tumors; IDH mutation status was not determined. All MRI scans were visually inspected by M.S. as previously described. An overview of the MRI settings is listed in the Supplementary Materials, Appendix 1. All tumors were semi-automatically segmented by M.S. on the T2-weighted scans using ITKSnap. Since the T1-weighted and T2-weighted scans were already co-registered in this study, the segmentation could be directly used for the T1-weighted scans without the need for registration. Brain masks were made using FSL's BET tool, with the same settings as for the EMC/HMC dataset.

### Classification algorithm

To predict the 1p/19q status of the tumors based on MRI features, the PREDICT toolbox was used. This toolbox was used to extract a total of 78 image features (such as image intensity, tumor texture, tumor shape, and tumor location) from the T1-weighted and T2-weighted MR image. These features, as well as the age and sex of the patient, were then used to train a Support Vector Machine (SVM), resulting in a total of 80 features. All parameter optimization and classifier training were performed on the EMC/HMC training set dataset using 100 iterations of stratified random-split cross-validation, with 80% of the data set used for training and 20% used for validation. Once the algorithm was optimized, no more

changes were made to the algorithm and it was then evaluated on the TCIA dataset. To evaluate the algorithm, the accuracy, sensitivity (1p/19q co-deletion prediction), specificity (1p/19q intact prediction), area under the receiver-operating-characteristic curve (AUC), weighted F1-score, and precision were determined by comparing the predicted labels with the reference labels obtained from tissue diagnosis. Full details of the algorithm can be found in the Supplementary Materials, Appendix 2 with more information about the evaluation metrics in Appendix 3. An overview of the classification algorithm is provided in Supplementary Materials, Online Figure 1.

To minimize the variance due to randomness in the algorithm training, an ensemble of 5 SVMs, which averages the predictions of the 5 independently trained models, was also constructed; the details can be found in Supplementary Materials, Appendix 2. One hundred different ensembles were constructed, and were evaluated on the TCIA dataset using the evaluation metrics described previously. Mean and standard deviation of the metrics over the 100 ensembles were computed.

To evaluate the contribution of the different features to the final prediction, a sensitivity analysis using polynomial chaos expansions was performed, resulting in Sobol indices for each feature.<sup>(25)</sup> The total Sobol index was used to determine the relative feature importance of the individual features. The total Sobol index is relative measure of the sensitivity of the algorithm to the input features. The OpenPC toolbox was used to create the polynomial chaos expansions and to calculate the Sobol indices.<sup>(26,27)</sup>

We also determined which patients from the TCIA dataset were considered as representative examples for the 1p/19q co-deleted and 1p/19q intact class by the algorithm. This was achieved by counting the number of times the algorithm correctly predicted the class for a specific patient in the 100 ensembles that were constructed. We evaluated the performance of the algorithm when the EMC/HMC and TCIA dataset were mixed instead of used as a separate train and validation set, to evaluate the effect of adding additional training data.

### **Prediction of 1p/19q status by clinical experts**

To compare the results of the algorithm with expert performance, the 1p/19q status of the TCIA tumors was also predicted by two neuroradiologists and two neurosurgeons at the Erasmus MC Brain Tumor Center. They were presented with the T1-weighted and T2-weighted images side by side for each patient as well as the sex and age to ensure that the algorithm and the raters had access to the same information. For each tumor the rater was then asked to choose whether they thought it was 1p/19q co-deleted or not, and to provide a confidence score ranging from 1 to 5 (1 indicating very unsure and 5 indicating very sure). This confidence score was then turned into a prediction 'score' by dividing it by 5 and multiplying it by 1 if the predicted label was co-deleted or by -1 if the predicted label was not co-deleted. In this way an AUC could be determined for the

manual classification. The accuracy, sensitivity, and specificity were determined in the same way as for the algorithm.

### Statistical analysis

Statistical analyses to test differences between the two datasets were performed with SPSS 21.0 statistical software (IBM Corp.). We tested whether the two datasets differed significantly from each other using the Mann–Whitney U test for continuous, non-normally distributed variables (age and volume) and the chi-squared test for all other categorical variables (sex, genetic analysis, presence of mild enhancement, codeletion status). Predictive performances (mean, 95% confidence interval (CI)) between the EMC/HMC training set and TCIA validation set were tested with the Welch t-test. Accuracy between the clinical experts and the algorithm were tested with the McNemar test. A p-value of  $< 0.05$  was considered statistically significant. The 95% CIs were calculated such that if the entire experiment of training on EMC/HMC and prediction on TCIA would be repeated, in 95% of the repetitions the result would lie within that interval.

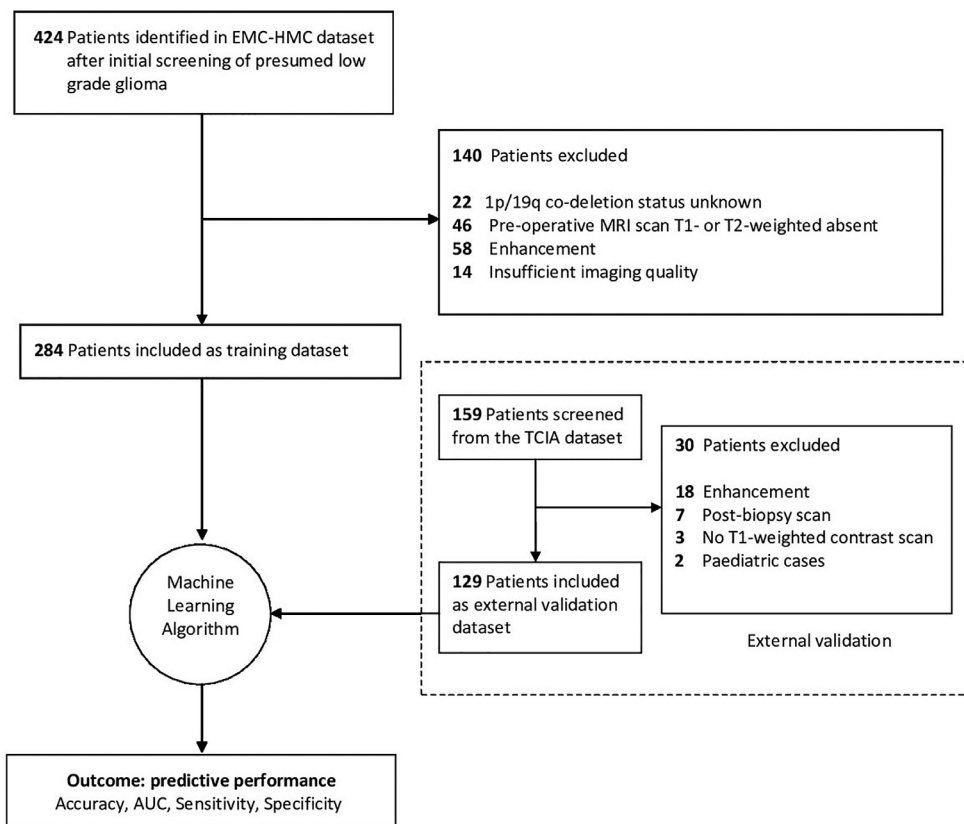
### Data Sharing

The data used in this study is available on Mendeley Data (<http://dx.doi.org/10.17632/rssf5nxxby.1>). The code for the construction and evaluation of the prediction algorithm is available on GitHub (<https://github.com/Svdvoort/PREDICT>). The code used to construct the polynomial chaos expansions and calculate the Sobol indices is available on GitHub as well (<https://github.com/Svdvoort/OpenPC>).

### Results

In the EMC/HMC dataset, 424 LGG were identified and screened for eligibility. Cases were excluded due to unknown 1p/19q co-deletion status (N=22), absence of T1- and/or T2-weighted MRI scans (N=46), enhancement (N=58), and unacceptable image quality (N=14), which resulted in 284 patients included for final analysis (Flowchart, Figure 1).

From the TCIA database, all 159 patients were screened for eligibility. Patients were excluded because of enhancement (N=18), signs of prior biopsy/surgical procedure (N=7), no post-contrast T1-weighted imaging available (N=3), and patients being younger than 18 years (N=2), resulting in 129 patients included in the external validation dataset (Flowchart, Figure 1). An overview of the excluded patients from the TCIA database as well as the reason for exclusion is available as Supplementary Material Appendix 4.

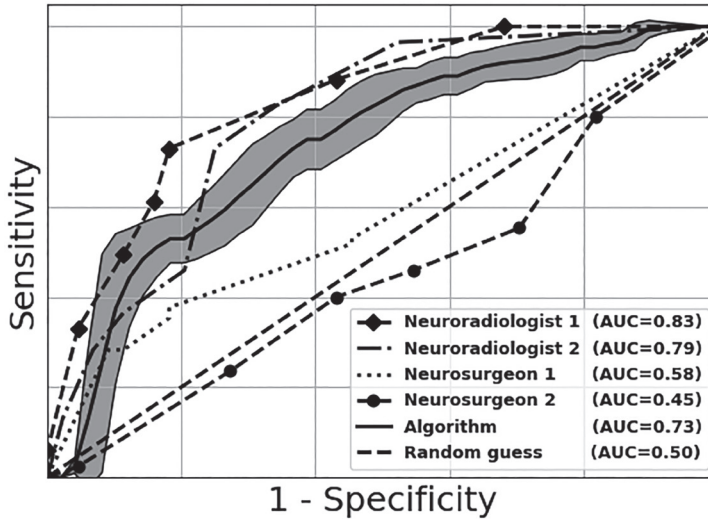


**Figure 1.** Flow diagram of the inclusion procedure for both the EMC/HMC training dataset and TCIA validation dataset.

There was no significant difference between the EMC/HMC and TCIA datasets for median age (43.0 years, 17.0 interquartile range (IQR) vs. 39 years, IQR 19.5 respectively;  $p=0.11$ ) and sex distribution (56.7% vs 52.7% male respectively;  $p=0.45$ ). Median tumor volume in the EMC/HMC dataset was significantly larger than in the TCIA dataset (median 47.80 cm<sup>3</sup>, IQR 58.65 vs. median 35.70 cm<sup>3</sup>, IQR 49.10),  $p=0.04$ ). There were fewer 1p/19q co-deleted tumors in the EMC/HMC compared with the TCIA dataset (35.20% vs. 65.40%,  $p<0.0001$ ). Patient and tumor characteristics of both datasets are further presented in Table 1.

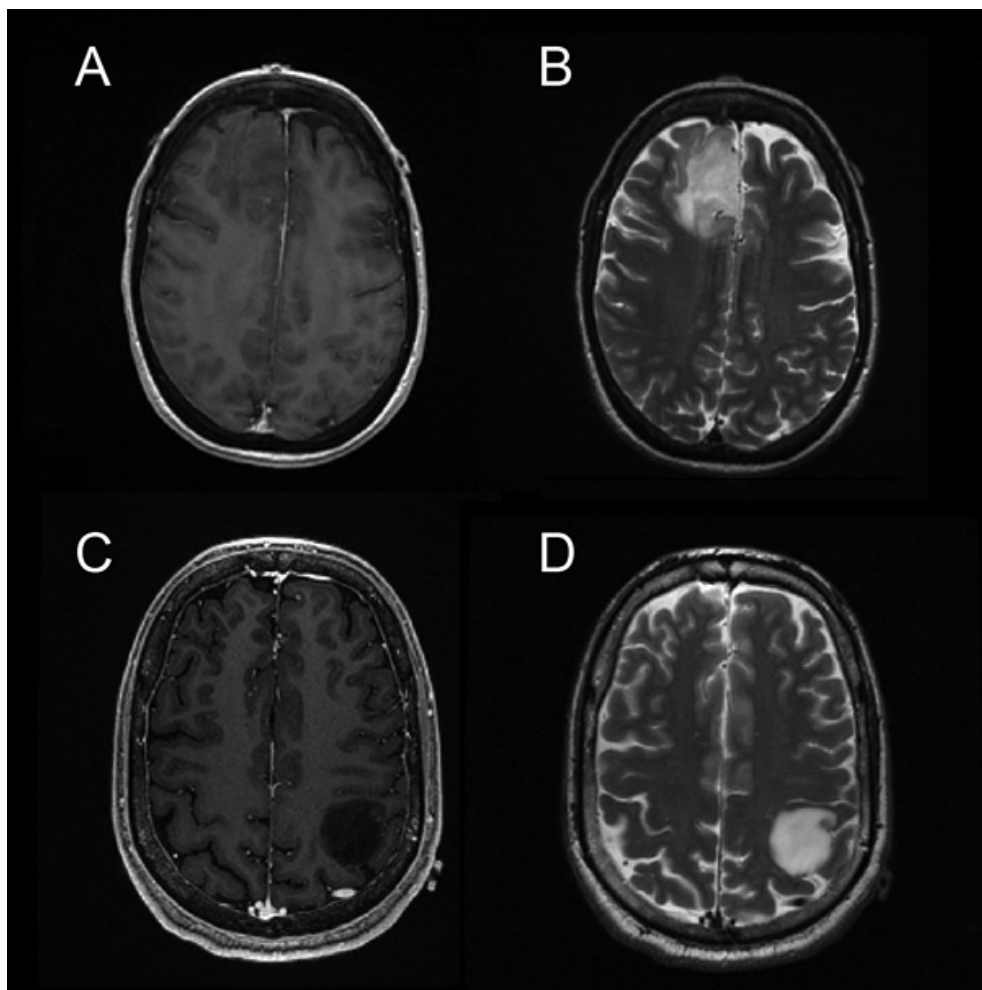
The predictive performance of the algorithm on the EMC/HMC training dataset, obtained from the cross validation, and the TCIA validation dataset is given in terms of accuracy, AUC, F1-score, precision, sensitivity and specificity in Table 2. The accuracy, AUC and sensitivity did not differ significantly between training and validation datasets ( $p=0.886$ ,  $p=0.746$ ,  $p=0.146$  respectively), while the specificity was significantly lower in the validation dataset ( $p=0.038$ ). The predictive performances of the clinical experts compared to the algorithm can be found in Table 3, and their ROC curves in Figure 2.

The algorithm had a higher AUC when compared with the average performance of the neurosurgeons, but a lower AUC when compared with the neuroradiologists. There was high variability in predictive performance between the clinical experts (AUC 0.449 - 0.830).



**Figure 2.** ROC curves of clinical expert and algorithm performance. For the performance of the algorithm the 95% confidence interval is plotted as well, representing the uncertainty due to randomness in model training.

The results of mixing the EMC/HMC dataset and the TCIA dataset are shown in Appendix 5. Mixing the datasets leads to a slightly improvement performance, but still within the confidence interval of the EMC/HMC dataset cross-validation results. According to the algorithm, the most important features for accurate 1p/19q co-deletion status prediction were the cranial/caudal location of the tumor, the skewness of the T2-weighted signal intensity (SI) histogram and one of the texture features, together with age and sex (Supplementary Materials, Online Figure 2). The algorithm identified a typical 1p/19q codeleted glioma as a frontal heterogeneous tumor as seen on T1-weighted and T2-weighted scans, while a typical 1p/19q intact glioma was identified as a parietal homogenous tumor, as shown in Figure 3.



**Figure 3.** (A) and (B) show a frontally located glioma. It is non-enhancing on post-contrast T1-weighted MRI (A). A heterogeneous signal intensity with indistinct border is visible on the T2-weighted MRI (B). Correctly predicted as a 1p/19q codeleted glioma (oligodendroglioma) by the algorithm. (C) and (D) show a parietally located glioma. It is non-enhancing on post-contrast T1-weighted MRI (C). A homogeneous signal intensity with sharply demarcated border is visible on the T2-weighted MRI (D). Correctly predicted as a 1p/19q intact glioma (astrocytoma) by the algorithm.



**Table 1.** Patient and tumor characteristics.

	EMC/HMC - Training Set (n = 284)	TCIA - Validation Set (n = 129)	P value
<b>Clinical</b>	n (%)	n (%)	
Age median, [IQR] in years	43 [17]	39 [19.5]	0.11
<b>Sex</b>			0.45
Male	161 (56.7)	68 (52.7)	
Female	123 (43.3)	61 (47.3)	
<b>Imaging</b>			
Volume median, [IQR] in cm <sup>3</sup>	47.8, [58.7]	35.7, [49.1]	0.04
Mild Enhancement			0.005
Yes	27 (9.5)	25 (19.4)	
No	257 (90.5)	104 (80.6)	
<b>Genetic</b>			
Histopathology (WHO 2016)			< 0.0001
Oligodendroglioma	100	85	
Astrocytoma	181	44	
Glioblastoma	3	0	
Method of Analysis			< 0.0001
NGS	214 (75.4)	0 (0)	
FISH	45 (15.8)	129 (100)	
MLPA	25 (8.8)	0 (0)	
1p/19q codeletion			<0.0001
Yes	100 (35.2)	85 (65.9)	
No	184 (64.8)	44 (34.1)	
IDH mutation			n/a
Yes	214 (75.4)	0 (0.0)	
No	35 (12.3)	0 (0.0)	
Unknown	35 (12.3)	129 (100.0)	

EMC Erasmus MC, HMC Haaglanden Medical Center, TCIA The Cancer Imaging Archive, NGS Next Generation Sequencing, FISH Fluorescent in situ hybridization, MLPA Multiplex Ligation Probe Assay, WHO World Health Organization.

**Table 2.** Predictive performances of the algorithm on the EMC/HMC training and TCIA validation datasets. The performances on the EMC/HMC training dataset were obtained by cross-validation, the performances on the TCIA validation dataset were obtained by training on the EMC/HMC dataset and then testing on the TCIA dataset.

	EMC/HMC - Training Set Mean (95% CI)	TCIA - Validation Set Mean (95% CI)	P value
<b>Accuracy</b>	0.698 (0.636 - 0.760)	0.693 (0.657 - 0.729)	0.872
<b>AUC</b>	0.755 (0.694 - 0.817)	0.723 (0.708 - 0.737)	0.313
<b>F1-score</b>	0.701 (0.640 - 0.761)	0.697 (0.661 - 0.733)	0.896
<b>Precision</b>	0.570 (0.491 - 0.649)	0.787 (0.754 - 0.820)	< 0.001
<b>Sensitivity</b>	0.657 (0.562 - 0.752)	0.732 (0.689 - 0.775)	0.123
<b>Specificity</b>	0.721 (0.628 - 0.813)	0.617 (0.544 - 0.691)	0.027

AUC: area under the curve.

**Table 3.** Predictive performance of four clinical experts compared with the algorithm on the TCIA Validation Dataset.

	Neuro- surgeon 1	Neuro- surgeon 2	Average of surgeons	Neuro- radiologist 1	Neuro- radiologist 2	Average of radiologists	Algorithm
<b>Accuracy,</b> with p value*	0.520, 0.073	0.457, 0.002	0.489	0.690, 0.720	0.574, 0.266	0.632	0.693
<b>AUC</b>	0.580	0.449	0.515	0.830	0.792	0.811	0.723
<b>Sensitivity</b>	0.370	0.459	0.415	0.610	0.459	0.535	0.732
<b>Specificity</b>	0.820	0.455	0.638	0.840	0.795	0.818	0.617

\* statistical comparison (McNemar) of accuracy between clinical experts and algorithm. AUC: area under the curve.

## Discussion

In this study, we developed an algorithm that predicted the 1p/19q co-deletion status of presumed LGG non-invasively based on preoperative MRI scans with an AUC of approximately 0.75. We tested the algorithm on an external, independent validation dataset. To the best of our knowledge, this is the first time this has been done in presumed LGG, and thus sets a benchmark for the expected performance in the real-world clinical setting. The algorithm had a higher AUC than the averaged AUC of the neurosurgeons, but lower than the averaged AUC of the neuroradiologists.

To the best of our knowledge, this is the first study performing a radiogenomics based machine learning study in LGG from the perspective of real-world clinical practice: we included all patients with presumed, non-contrast enhancing LGG, rather than a selection of histopathological defined LGG patients. This is important, since in a clinical setting the genetic mutation is unknown at first symptomatic presentation. Since it is only known after surgery and molecular analysis, we aimed to mirror this real-world situation as best as possible by not selecting patients based on histological tumor features, but on the imaging features that are available at the time of presentation. Note that subsequently all lesions were surgically resected to obtain the ground truth data based on confirmed

histological and molecular analysis. We trained the algorithm on a heterogeneous training dataset and used a separate, completely independent, publicly available dataset with data from an entirely different institute to validate the algorithm. As such, this study is the first to demonstrate that the performance of a radiogenomics algorithm in predicting the 1p/19q co-deletion status of presumed LGG based on MRI scans was robust and matched expert clinical performance. Furthermore, we were also able to show which image features were important in the classification, increasing the clinical understanding of the machine learning algorithm and potentially aiding better acceptance, as well as furthering fundamental research into understanding of glioma pathophysiology.

Although other studies did already investigate the non-invasive prediction of the molecular subtype of LGG, these often focused on IDH mutations only and did not consider the 1p/19q co-deletion status.<sup>11,28,29</sup> In comparison with studies that did look at the 1p/19q co-deletion, we used a larger cohort and an external validation dataset,<sup>10,13,14,15,30,31</sup> which makes our results more robust and generalizable respectively. Although one study did use an independent dataset, this study used only five patients to externally validate the 1p/19q co-deletion predictive performance of the algorithm, which severely limits the reliability of its predictive performance.<sup>12</sup> Additionally, that specific study retrospectively selected patients with histopathological defined LGG only, which represents the diagnosis-treatment workflow in clinical practice less accurately. The starting point of decision making on the optimal treatment strategy for LGG is the initial diagnosis on first MRI, when a non-contrast enhancing space occupying lesion is seen, at which point knowledge on the histopathological grade is not yet available.

The optimal timing and effect of surgical treatment of LGG is extensively being debated within literature and has recently been re-evaluated in the light of molecular subclassification after the introduction of WHO 2016 criteria.<sup>5,6,32,33</sup> Currently, the molecular subtype based on 1p/19q codeletion and IDH mutation can be diagnosed only after obtaining tissue with biopsy or surgery. Indeed, as our results suggests, it is even for experienced neuro-oncological surgeons and radiologists a challenge to accurately predict the codeletion status of non-enhancing tumors based on preoperative MRI scans (AUC 0.45-0.83).

There are two scenarios in which preoperative, non-invasive prediction of the 1p/19q codeletion status based on MRI would be clinically relevant. First, some patients are not eligible for surgical resection or diagnostic biopsy due to older age, poor neurological condition, or tumor localization in eloquent brain areas or basal ganglia.<sup>33</sup> However, knowledge of the molecular LGG subtype might add to a more appropriate (timing of) chemo- and/or radiotherapy regimes (immediate post-operative therapy vs watchful waiting).<sup>34</sup> Therefore, non-invasive, accurate prediction of the molecular subtype on imaging could help clinicians to select the optimal treatment when tissue diagnosis is difficult to obtain. Second, it is suggested that post-surgical residual 1p/19q intact, IDH mutated tumor has a more negative impact on survival than residual 1p/19q co-deleted,

IDH mutated (oligodendroglioma) tumor.<sup>5,6</sup> With pre-surgical knowledge of the specific molecular subtype the surgeon can make a better informed decision on whether or not to push the limits of resection at the time of surgery, avoiding on the one hand re-resection in case of residual 1p/19q intact, IDH mutated tumor and less-justified post-surgical deficits in 1p/19q co-deleted tumor on the other hand. Clearly, the diagnostic accuracy of our algorithm is as yet too low to rely on for clinical practice. However, the results are promising because they generalize through multiple datasets, encouraging future research in this direction.

Our study had a few limitations. First, for the current study only the T1-weighted and T2-weighted images were used, while diffusion weighted and perfusion imaging also contain relevant features for the 1p/19q status. These sequences were not included in the development of the present algorithm, as these were scarcely available in both datasets.

Second, the IDH mutation status was undetermined in all of the TCIA cases and in 35 cases of the EMC/HMC dataset. Since molecular subclassification according to the WHO 2016 guidelines is based on both the 1p/19q co-deletion status and IDH mutation status, it is important to predict both. Therefore, for our future work, we are expanding our database with more patients in whom the tumor IDH status is known, to eventually be able to predict all clinically relevant subtypes of presumed LGG. There was an imbalance between the EMC/HMC dataset and the TCIA dataset in terms of the number of co-deleted and intact cases. Despite this imbalance, our algorithm still shows similar performance between the cross-validation result of the EMC/HMC dataset and the performance on the TCIA test dataset.

## **Conclusion**

Our results suggest that our algorithm can non-invasively predict the 1p/19q co-deletion status of presumed LGG with a performance that in general outperforms oncological neurosurgeons. We evaluated our algorithm on an independent, multicenter dataset, which demonstrated that our algorithm is robust and generalizable. The prediction of the 1p/19q co-deletion status by our algorithm can eventually add value to clinical decision making by tailoring the treatment strategy for patients with presumed LGG even prior to surgery.

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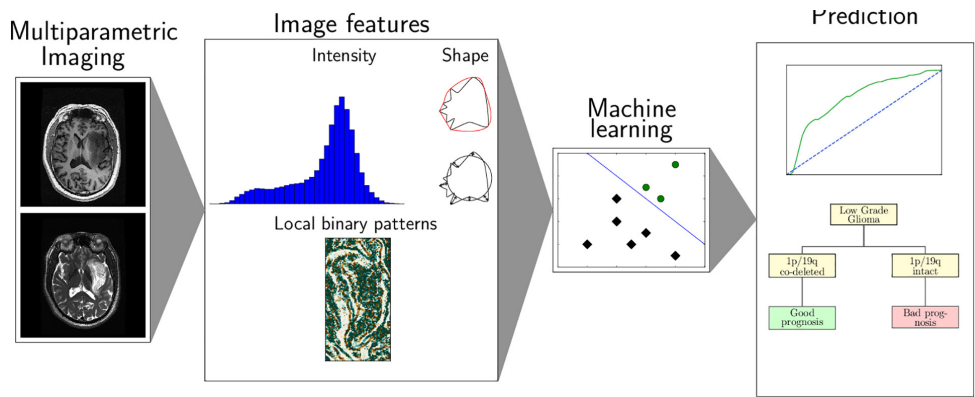
Supplementary materials

Appendix 1

Overview of MRI settings from the EMC/HMC and TCIA datasets.

MRI Setting	Training dataset (EMC/HMC)		Validation dataset (TCIA)	
	T1-weighted min-max	T2-weighted min-max	T1-weighted min-max	T2-weighted min-max
Voxel spacing in-plane (mm)	0.38 x 0.38 - 1.13 x 1.13	0.23 x 0.23 - 1.02 x 1.02	0.47 x 0.47 - 1.1 x 1.1	0.43 x 0.43 - 1.1 x 1.1
Matrix Size	256 x 176 - 1024 x 307	256 x 224 - 1024 x 1024	256 x 256 - 512x512	256 x 256 - 512x512
Echo Time (ms)	1.7 - 20	79.2 - 379	2.6 - 21	12.3 - 108.3
Repetition Time (ms)	3.8 - 1940	2000 - 13468.5	8.2 - 983.3	2033.3 - 8116.6
Slice Thickness (mm)	0.9 - 7.2	1.0 - 7.2	1.0 - 5.0	2.0 - 5.0
Number of Slices	19 - 248	19 - 304	20 - 196	20 - 84
Field Strength (Tesla)	0.5, 1.5 or 3.0	0.5, 1.5 or 3.0	1.5 or 3	1.5 or 3
# of 2D scans	147	264	33	129
# of 3D scans	137	20	96	0

Appendix 2



The radiogenomics algorithm.

An in-house radiomics pipeline, ‘PREDICT’, was used for the prediction of the 1p/19q co-deletion status (<https://github.com/Svdvoort/PREDICT>). This pipeline takes the T1- and T2-weighted MR images, tumor and brain segmentations on both scans, and labels indicating 1p/19q co-deletion status for each patient which can then be used to train a support vector machine (SVM)<sup>(1)</sup>. This SVM can then be used to predict the labels for unseen images. In this appendix the different steps of the pipeline are explained.



## Data pre-processing

First, the segmentations were transformed to the T2-weighted scan and the T1-weighted scans. If the segmentation was done on T2w-FLAIR, the T2w-FLAIR scan was registered to the T2-weighted scan using SimpleElastix with an affine transform by maximisation of mutual information.<sup>(18)</sup> The computed transform was used to map the T2w-FLAIR segmentation to the T2-weighted scan. Then, the T2-weighted scans were registered to the T1-weighted scans. The computed transformation was used to transform the segmentation from the T2-weighted scans to the T1-weighted scan. This method was applied to both the segmentations originally done on the T2-weighted scans, and the ones that were registered to the T2-weighted scan from the T2w-FLAIR. All segmentations and registrations were checked by M.S., and manual adjustments of the segmentations were made by F.I. if necessary.

The next step of the pre-processing was to obtain the brain masks, which were created by FSL BET<sup>(11)</sup> with a setting of 0.5. The brain masks were used to normalize the scans. This was done by extracting the intensity values that lie within the brain mask, after which Z-scoring was applied. In this way, the mean intensity within the brain mask was 0 and the standard deviation of the intensities within the brain mask 1. These pre-processed images were then processed by the next step of the algorithm to extract the features.

## Features

In total 80 features were used in our algorithm: 78 features from the T1-weighted and the T2-weighted images, and age and sex. These features were split into 5 groups: tumor intensity, tumor texture, tumor shape, tumor location, and demographic features. All of these features were only extracted within the tumor mask.

Image intensity was described using 11 features: minimum, maximum, mean, median, standard deviation, skewness, kurtosis, range from the 2nd to 98th percentile, range from the 25th to 75th percentile (the quartile range), the mode position (the bin in the intensity histogram which had the highest occurrence) of the intensity and the energy defined as:

$$E = \sum \left( (I + \min(I))^2 \right), \text{ where } I \text{ is the image intensity}$$

These features were calculated over the entire tumor in 3D. Rotation invariant Local Binary Patterns (LBPs) were used to describe the texture of the tumor<sup>(2)</sup>. The advantage of LBPs is that they are grayscale invariant, meaning that not the intensity values themselves, but the differences between intensity values of different voxels are taken into account. This is an advantage when using MRI scans, because in weighted MRI scans the absolute values signal intensities themselves do not carry information, it is only the difference in gray values in the image. LBP features were determined for a radius of 1, 3 and 5 with 8, 24 and 24 points, respectively. The LBP was determined for each slice of the MRI scan in

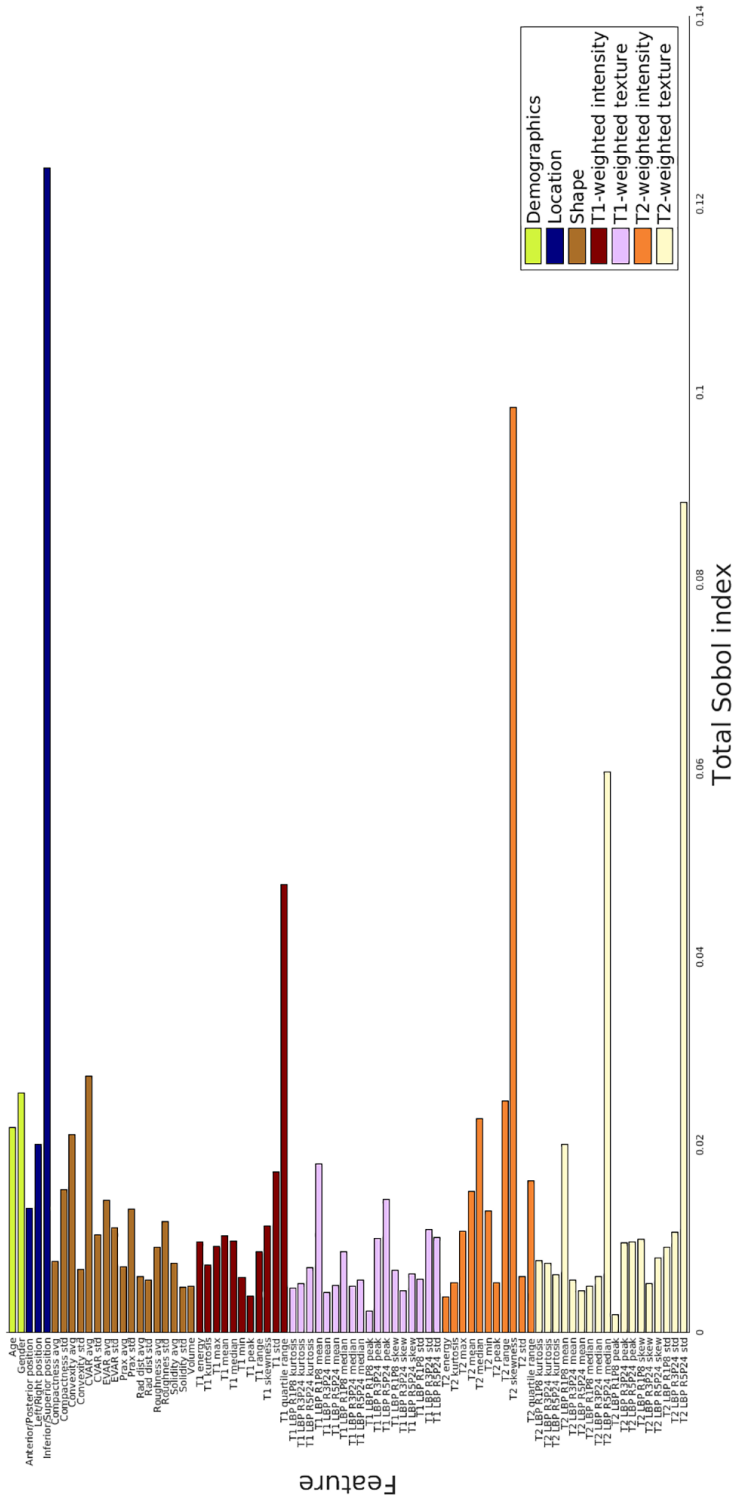
which tumor was present. The results over all pixels within the tumor and slices containing tumor were then concatenated and consequently the mean, standard deviation, median, kurtosis and skewness, and peak value were calculated for each setting, resulting in 18 LBP features. The intensity features and the texture features were calculated on both the T1-weighted and T2-weighted scan, resulting in 58 features.

Shape features were derived from the segmentation on the T2-weighted image. Shape features consisted of the compactness, radial distance, roughness, convexity, circular variance (cvar), ratio of principal axes (prax), elliptical variance (evar) and solidity<sup>(4,5)</sup>. All of these features were calculated on a per-slice basis (only for slices that contained tumor), after which the mean and standard deviation of the features over the slices were included as features in the algorithm, resulting in 16 features. The volume was also taken as a 3D shape feature, resulting in 17 shape features.

Tumor location was determined by using the center of mass (COM) from the brain mask and COM of the tumor segmentation. The anterior/posterior, left/right and inferior/superior coordinate of the vector pointing from the COM of the brain to the COM of the tumor were then included as features, resulting in 3 location features. Patient age and patient sex were included as the demographic features.

An overview of all the features is given in Table 1, along with the Imaging Biomarker Standardization Initiative (IBSI) code when the feature matched with one included in the IBSI<sup>(6)</sup>. Many of our features are based on local binary patterns, which are currently considered outside the scope of IBSI. However, preliminary experiments have indicated that including these features improves the performance of the classifier (results not shown). Z-scoring was applied to the features (normalized such that the mean of the features was 0 and the standard deviation was 1). This was done based only on the training set; the resulting normalisation settings were saved to apply the same normalisation to the validation set.

### Appendix 3.



**Table 1.** Overview of the features included in the algorithm.

Modality	Slice averaging	Feature	IBSI code
N/A	N/A	Age	N/A
N/A	N/A	Gender	N/A
N/A	N/A	Anterior/Posterior position	None
N/A	N/A	Left/Right position	None
N/A	N/A	Inferior/Superior Position	None
Segmentation	Average	Compactness	KRCK
Segmentation	Standard deviation	Compactness	KRCK
Segmentation	Average	Convexity	None
Segmentation	Standard deviation	Convexity	None
Segmentation	Average	Circular variance	None
Segmentation	Standard deviation	Circular variance	None
Segmentation	Average	Elliptic variance	None
Segmentation	Standard deviation	Elliptic variance	None
Segmentation	Average	Ratio of principal axis	None
Segmentation	Standard deviation	Ratio of principal axis	None
Segmentation	Average	Radial distance	None
Segmentation	Standard deviation	Radial distance	None
Segmentation	Average	Roughness	None
Segmentation	Standard deviation	Roughness	None
Segmentation	Average	Solidity	Inverse of 7T7F
Segmentation	Standard deviation	Solidity	Inverse of 7T7F
Segmentation	None	Volume	2PR5
T1	None	Energy	Similar to N8CA, except min intensity is added
T1	None	Kurtosis	IPH6
T1	None	Max	84IY
T1	None	Mean	Q4LE
T1	None	Median	Y12H
T1	None	Min	1GSF
T1	None	Peak	None
T1	None	Range	Similar to 20JQ, except based on 2nd and 98th percentile
T1	None	Skewness	KE2A
T1	None	Standard deviation	Square root of ECT3
T1	None	Quartile range	SALO
T1	None	LBP R1P8 Kurtosis	None
T1	None	LBP R3P24 Kurtosis	None

T1	None	LBP R5P24 Kurtosis	None
T1	None	LBP R1P8 Mean	None
T1	None	LBP R3P24 Mean	None
T1	None	LBP R5P24 Mean	None
T1	None	LBP R1P8 Median	None
T1	None	LBP R3P24 Median	None
T1	None	LBP R5P24 Median	None
T1	None	LBP R1P8 Peak	None
T1	None	LBP R3P24 Peak	None
T1	None	LBP R5P24 Peak	None
T1	None	LBP R1P8 Skewness	None
T1	None	LBP R3P24 Skewness	None
T1	None	LBP R5P24 Skewness	None
T1	None	LBP R1P8 Standard deviation	None
T1	None	LBP R3P24 Standard deviation	None
T1	None	LBP R5P24 Standard deviation	None
T2	None	Energy	Similar to N8CA, except min intensity is added
T2	None	Kurtosis	IPH6
T2	None	Max	84IY
T2	None	Mean	Q4LE
T2	None	Median	Y12H
T2	None	Min	1GSF
T2	None	Peak	None
T2	None	Range	Similar to 20JQ, except based on 2 <sup>nd</sup> and 98 <sup>th</sup> percentile
T2	None	Skewness	KE2A
T2	None	Standard deviation	Square root of ECT3
T2	None	Quartile range	SALO
T2	None	LBP R1P8 Kurtosis	None
T2	None	LBP R3P24 Kurtosis	None
T2	None	LBP R5P24 Kurtosis	None
T2	None	LBP R1P8 Mean	None
T2	None	LBP R3P24 Mean	None
T2	None	LBP R5P24 Mean	None
T2	None	LBP R1P8 Median	None
T2	None	LBP R3P24 Median	None
T2	None	LBP R5P24 Median	None
T2	None	LBP R1P8 Peak	None
T2	None	LBP R3P24 Peak	None

T2	None	LBP R5P24 Peak	None
T2	None	LBP R1P8 Skewness	None
T2	None	LBP R3P24 Skewness	None
T2	None	LBP R5P24 Skewness	None
T2	None	LBP R1P8 Standard deviation	None
T2	None	LBP R3P24 Standard deviation	None
T2	None	LBP R5P24 Standard deviation	None

The IBSI column indicates the code of the feature if it is present in the IBSI. Abbreviations: IBSI: Imaging Biomarker Standardization Initiative, LBP: local binary pattern.

### Over-sampling of the minority class

There was an imbalance in the training dataset as there were more examples of 1p/19q intact than of 1p/19q co-deleted tumors. As a result, it was more difficult for the algorithm to correctly predict 1p/19q co-deleted tumors within the training dataset. To (partially) solve this problem we used Synthetic Minority Over-sampling Technique (SMOTE). SMOTE allows for the oversampling of the minority class and synthetically increased the number of samples for the 1p/19q co-deleted class<sup>(7)</sup>. Here, an SVM with a polynomial kernel was used to create the synthetic examples, with a ratio of 1 (meaning that there will be an equal number of samples from both classes) using 5 neighbours.

### Classification

Support Vector Machines (SVMs) were used as classification algorithm<sup>(7)</sup>. SVMs were constructed using scikit-learn<sup>(8)</sup> with a polynomial kernel. The polynomial kernel was defined as:

$$K(X, Y) = (\gamma < X, Y > + C_0)^P$$

SVMs were constructed with a maximum of  $1 \cdot 10^7$  iterations. The hyperparameters of the SVM were optimized using a 5-fold cross-validation where 20% of the training dataset was used as an internal validation set. A random search of 50,000 iterations was used to find the optimal hyperparameters. This optimization search for the optimal  $C$ , the regularization parameter of the SVM, as well as three parameters of the polynomial kernel:  $\gamma$ , which defines how much each sample is weighted,  $C_0$ , a trade-off between high-order and low-order terms, and  $P$ , the order of the polynomial kernel. The distributions for the parameters are presented in Table 1.

**Table 2.** Overview of the range of hyperparameters used in the optimization of the SVM.

Parameter	Lower bound	Upper bound
C	0	$1 \cdot 10^{-6}$
P	1	7
$C_0$	0	$1 \cdot 10^{-3}$
	$1 \cdot 10^{-5}$	1

SD standard deviation, IQR Inter Quartile Range, CE contrast enhancement, FLAIR Fluid-attenuated inversion recovery.

The optimal hyperparameters were chosen based on the settings that gave the highest average area under curve over the 5 folds. Using these optimal hyperparameters, the final SVM was then constructed using the complete training dataset.

### Ensemble SVM

To increase the predictive performance and minimize the variability of the predictions, an ensemble of SVMs was constructed. Five trained SVMs were taken to form the ensemble. All 5 SVMs were then used to predict a test sample. The posteriors of the SVMs were averaged for the sample. Based on this averaged posterior the final label was determined (positive class if posterior  $\geq 0.5$ , negative class if posterior  $< 0.5$ ). To form the 100 ensembles 500 SVMs had to be trained. No single SVM was used in two different ensembles: all ensembles were completely unique. The resulting 100 ensemble SVMs could then be used to make a prediction of the 1p/19q status for a new sample.

## Appendix 3

### Evaluation metrics

The accuracy, area under the Receiver Operating Characteristic (ROC) curve (AUC), weighted F1-score, precision, sensitivity, and specificity were calculated to evaluate the performance of the algorithm and clinical experts. These were based on the variables extracted from the confusion matrix:

- True Positive (TP): number of true 1p/19q co-deleted predicted as 1p/19q co-deleted
- False Positive (FP): number of true 1p/19q intact predicted as 1p/19q co-deleted
- True Negative (TN): number of true 1p/19q intact predicted as 1p/19q intact
- False Negative (FN): number of true 1p/19q co-deleted predicted as 1p/19q intact

The evaluation metrics were then defined as follows:

$$\text{Accuracy} = \frac{TP + TN}{TP + FP + TN + FN}$$

$$\text{F1-score} = \frac{TP^2 + TP \cdot FN + FP \cdot TN + TN^2}{TP + FN + FP + TN}$$

$$\text{Precision} = \frac{TP}{TP + FP}$$

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Specificity} = \frac{TN}{TN + FP}$$

The ROC curve and AUC were obtained using scikit-learn.

## Appendix 4

Table of excluded patients from TCIA-LGG dataset.

Patient ID	Reason for Exclusion
LGG-223	Enhancement
LGG-234	Enhancement
LGG-241	Enhancement
LGG-254	Post-biopsy
LGG-260	Enhancement
LGG-282	Enhancement
LGG-295	Enhancement
LGG-296	Post-biopsy
LGG-307	Enhancement
LGG-310	Post-biopsy
LGG-313	Post-biopsy
LGG-334	Post-biopsy
LGG-338	Enhancement
LGG-354	Enhancement
LGG-365	Enhancement
LGG-367	Post-biopsy
LGG-377	Enhancement
LGG-387	Enhancement
LGG-500	Age < 18 years
LGG-506	Enhancement
LGG-532	Age < 18 years
LGG-545	No post-contrast T1-weighted image



LGG-558	No post-contrast T1-weighted image
LGG-561	Enhancement
LGG-563	No post-contrast T1-weighted image
LGG-594	Enhancement
LGG-600	Enhancement
LGG-601	Post-biopsy
LGG-634	Enhancement
LGG-642	Enhancement

## Appendix 5

### Results of mixing EMC/HMC and TCIA datasets.

We evaluated the effect of mixing the EMC/HMC and TCIA datasets, instead of using them as separate training and validation set. This was done using the same cross-validation approach as was used for the EMC/HMC dataset cross-validation. In this case all 413 patients were pooled together. Then 100 iterations of stratified random-split cross-validation with 80% of the dataset used for training and 20% of the dataset used for validation was performed. The results are shown in Table 1.

**Table 1.** Predictive performances of the algorithm on the mixed EMC/HMC and TCIA dataset.

	Cross-validation on combined EMC/HMC and TCIA dataset	Cross-validation on EMC/HMC dataset
	Mean (95% CI)	Mean (95% CI)
Accuracy	0.717 (0.670 – 0.764)	0.698 (0.636 - 0.760)
AUC	0.780 (0.729 – 0.830)	0.755 (0.694 - 0.817)
F1-score	0.717 (0.670 – 0.763)	0.701 (0.640 - 0.761)
Precision	0.676 (0.620 – 0.732)	0.570 (0.491 - 0.649)
Sensitivity	0.710 (0.637 – 0.783)	0.657 (0.562 - 0.752)
Specificity	0.723 (0.658 – 0.788)	0.721 (0.628 - 0.813)

The results for the combined EMC/HMC and TCIA dataset show a slight improvement over the cross-validation on the EMC/HMC dataset alone but they are still within the confidence interval of the EMC/HMC dataset results, except for the precision. This shows that although there is a slight improvement, our algorithm is already quite robust and adding more data (from a different source) does not lead to a large increase in performance.

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# Chapter 4

## WHO 2016 subtyping and automated segmentation of glioma using multi-task deep learning

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# Chapter 5

Changes in language white matter tract microarchitecture are associated with cognitive deficits in patients with presumed low grade glioma

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*J Neurosurg. 2018 Jun 8:1-9*

## **Abstract**

### **Background**

The aim of this study was to determine whether cognitive functioning of presumed low-grade glioma patients is associated with white matter (WM) tract changes.

### **Methods**

We included 77 patients with presumed low-grade glioma who underwent awake surgery between 2005 and 2013. Diffusion tensor imaging (DTI) with deterministic tractography was performed pre-operatively to identify the arcuate (AF), inferior fronto-occipital (IFOF) and uncinate fasciculus (UF) and to obtain mean fractional anisotropy (FA) and mean diffusivity per tract. All patients were assessed pre-operatively with an extensive neuropsychological protocol, including the language, memory and attention/executive function domains. Linear regression models were used per cognitive domain and per DTI metric of the three WM tracts.

### **Results**

Significant correlations (corrected for multiple testing) were found between FA of the AF and the repetition test of the language domain ( $\beta = 0.59$ ,  $p < 0.0001$ ), and between FA of the IFOF and the imprinting test of the memory domain ( $\beta = -0.55$ ,  $p = 0.002$ ) and attention test of the attention and executive function domain ( $\beta = -0.62$ ,  $p = 0.006$ ).

### **Conclusion**

In glioma patients, language deficits in repetition of speech, imprinting and attention deficits are associated with changed microarchitecture of the arcuate fasciculus and the inferior fronto-occipital fasciculus.



## Introduction

Low grade gliomas (LGG) are World Health Organization (WHO) Grade I-II, slow growing, primary brain tumors that commonly show no or minimal contrast-enhancement. Patients often present with seizures, but headache and (mild) cognitive deficits are also frequently observed<sup>(31)</sup>. Due to a widespread representation of cognitive functions, it is important to make use of a neuropsychological protocol in glioma patients to detect deficits<sup>(24)</sup>. Satoer et al. showed with a neuropsychological assessment (NP) in a series of 45 glioma patients pre- and postoperative deficits in the domains of language, memory and the attention/executive functions<sup>(26)</sup>. We hypothesize that such cognitive deficits can – at least partly – be attributed to changes in white matter (WM) tract microarchitecture due to tumor effects such as edema or infiltration.

In vivo anatomical visualization of WM tracts, such as the arcuate fasciculus (AF), inferior fronto-occipital fasciculus (IFOF) and uncinate fasciculus (UF) can be obtained with the use of Diffusion Tensor Imaging (DTI) based tractography. DTI is a magnetic resonance imaging (MRI) modality in which the 3D diffusion of free water in the brain can be assessed. Since water diffuses preferentially along the direction of the white matter fibers instead of perpendicular to it, measuring the direction of free water diffusion allows for an approximation of the white matter fiber orientation. On a DTI-derived color-coded map, the white matter tracts are conventionally represented in green in the anterior-posterior direction (e.g. optical fibers), in red in the left-right direction (e.g. corpus callosum) and in blue in the ventral-dorsal direction (e.g. cortico-spinal tract). To delineate the specific white matter tracts, specific regions of interests (ROIs) are manually delineated on DTI images based on a priori anatomical knowledge. WM tracts of interest running through these ROIs are then visualized in 3D using tractography<sup>(5,32)</sup>. The AF is an association bundle that is involved in language by connecting perisylvian language areas of the frontal, temporal and parietal lobes<sup>(5)</sup>. Intraoperative stimulation of the AF elicits phonemic paraphasia<sup>(9)</sup>. The IFOF is an association bundle that connects the occipital lobe and the orbitofrontal cortex. The IFOF seems to be involved in several cognitive functions such as language (semantics and reading)<sup>(10)</sup>, but also attention and visual processing abilities<sup>(5,7)</sup>. The UF is an association bundle that connects the orbitofrontal cortex with the anterior temporal lobe. The UF is suggested to be involved in memory and language.<sup>(24)</sup>

Quantitative information on WM tract microarchitecture can be derived from tractography and expressed as fractional anisotropy (FA) and mean diffusivity (MD). FA is a measure of microstructural integrity with a value between 0 and 1, with 0 meaning the diffusion is the same in all directions (isotropic diffusion, such as seen in the ventricles) and close to 1 meaning diffusion of water is seen along a specific direction (anisotropic diffusion, such as seen along white matter tracts). A reduction of FA in a white matter tract is considered to be an indication of loss of tract integrity. MD is a measure of the average water diffusion and reflects membrane density. High MD is seen in free water

(the ventricles), and low MD in the white matter. An increase of MD in the white matter is considered an indication of the increase of the extracellular space, such as seen in edema. While derived from the same imaging sequence, FA and MD reflect different aspects of white matter tract structure, and can thus be used to assess the effects of tumor infiltration and edema.<sup>(2)</sup> Importantly, perilesional WM tracts and edematous zones around glioma show a reduction in FA values which suggests increased extracellular water and fiber disorganization due to tumor infiltration<sup>(1)</sup>. Studies have shown that tumor infiltrated WM tracts such as the AF have a relationship with language impairment<sup>(3,15)</sup>. However, to the best of our knowledge there is as yet no large-scale quantitative correlation of WM tract microarchitecture with cognitive functioning in LGG patients.

The purpose of this study was to determine whether cognitive functioning of presumed LGG patients is associated with changes in language WM tract microarchitecture. We analyzed FA and MD values of the AF, IFOF, UF in relation to three cognitive domains (language, memory and attention/executive functions) assessed with an extensive NP pre-operatively in patients selected for awake surgery.

## METHODS

### Study participants

A total of 131 patients (> 18 years) with presumed LGG who underwent awake surgery between April 2005 and May 2013 were considered for this retrospective study. Non-to-mildly contrast enhancing tumors, i.e. presumed LGG, on pre-operative MRI scan were considered. Note that if upon histopathological examination – *after* resection – such tumors displayed high grade glioma features, patients were retained in the study, to ensure that our findings are valid for the real-life clinical situation of pre-operative assessment and selection of patients, when histopathology is not yet available. Hence the use of the term *presumed* LGG, based on clinical and radiological characteristics. All patients were native Dutch speakers. Patients with recurrent glioma (n=24), without pre-operative NP (n=3), without sufficient DTI data (n=25), or a time frame between NP and DTI longer than 6 months (n=2) were excluded, resulting in a total of 77 patients available for data analysis (Table 1). Of the 77 patients (mean age 43 years, range 20-74), 49 were male (63.6%) with a mean age of 44 years (range 21 – 74), while all 28 females (36.4%) had a mean age of 40 years, (range 20 - 60). Although most tumors were localized in the left hemisphere, 15 patients with right-sided tumors were also included in this study, because they had right dominance for language as seen on fMRI, which was performed pre-operatively. Therefore, these patients were selected for awake surgery to prevent damage to the Broca region. The study was approved by the Medical Ethical Committee, who waived the need for written informed consent from the patients due to the retrospective nature of this study and the (emotional) burden that would result from contacting the patients or their relatives to obtain consent.

**Table 1.** Baseline patient characteristics.

Characteristics	N (%)	Characteristics	N (%)
<b>Age in years</b>		<b>Tumor localization</b>	
mean; range	43 ( 20 – 74 )	<b>Right</b>	15 (20.0)
<b>Gender</b>		Frontal	3 (3.9)
male/female	49 (63.6) / 28 (36.4)	Parietal	10 (13.0)
<b>Handedness</b>		Temporal	2 (2.6)
Right	54 (70.1)	Occipital	0 (0)
Left	5 (6.5)	<b>Left</b>	62 (80.0)
Unknown	18 (23.4)	Frontal	12 (15.6)
<b>Karnofsky Performance Status</b>		Parietal	23 (29.9)
100-90	69 (90)	Temporal	26 (33.8)
80-70	8 (10)	Occipital	1 (1.3)
<b>Tumor grade</b>		<b>Pathological tumor growth</b>	
Low grade glioma	47 (61)	Diffuse infiltrative	37 (48.1)
High grade glioma	30 (39)	Circumscribed	40 (51.9)
<b>Histopathological subtype</b>			
Oligodendroglioma WHO grade II	21 (27.3)		
Astrocytoma WHO grade II	13 (16.9)		
Oligoastrocytoma WHO grade II	11 (14.3)		
Oligodendroglioma WHO grade III	16 (20.8)		
Astrocytoma WHO grade III	12 (15.6)		
Oligoastrocytoma WHO grade III	3 (3.9)		
Glioblastoma WHO grade IV	1 (1.3)		

WHO = World Health Organization.

### Tumor localization and characterization

Tumor localization was determined by a neuroradiologist (8 years of experience) on pre-operative T1 and T2 weighted images. The histopathological type and grade of the tumor were determined by a neuropathologist from tissue obtained during tumor resection.

### Image acquisition and preprocessing

Diffusion MR data were acquired using a single shot spin echo planar imaging (EPI) sequence at 1.5T (n = 20) and 3.0T (n = 57) (GE Healthcare, Milwaukee, USA) with an eight-

channel head coil. In general, 25 non-collinear gradient directions at  $b = 1000 \text{ s/mm}^2$  ( $n = 71$ ) and 3 images at  $b = 0 \text{ s/mm}^2$  were acquired ( $n = 64$ ). In six patients, all imaged at 3.0T, 31 gradient directions were acquired with 4 images at  $b = 0 \text{ s/mm}^2$ . Slice thickness was 2.0–3.5 mm and in-plane resolution 1.9–3.4 mm<sup>2</sup>. Raw diffusion MRI data were transferred to an offline workstation. All images were first visually inspected for the presence of apparent artefacts. MRI data were pre- and post-processed using ExploreDTI<sup>(17)</sup>. Motion and eddy current correction of the DWI was not performed to avoid interpolation errors. Instead, the acquired native data and the data-quality-summary report were visually inspected in ExploreDTI. The gradient components in the x/y/z axes were manually checked and adapted if needed to the standard color-convention (left-right: red, top-bottom: blue, and front-back: green). Subsequently, the diffusion tensors were estimated using nonlinear least squares. The following thresholds were used to perform tractography: FA termination threshold of 0.2, angle threshold of 45°, step length: 0/5.

### Tractography

Deterministic tractography was used to identify the AF, IFOF and UF in the affected hemisphere according to standardized procedures<sup>(30)</sup>. From each of the reconstructed tracts, average FA and MD values were obtained. The rater was blinded for NP test results of the patients at time of tractography.

### Arcuate fasciculus

For the AF (Figure 1), a coronal slice was selected at the level of the primary motor cortex and the seed ROI was placed on the green (anterior-posterior) triangular shaped projections of the superior longitudinal fasciculus lateral to the blue projections of the cortical spinal tract in the posterior parietal lobe. For the target ROI, an axial slice was selected at the level of the genu of the corpus callosum. The blue (cranial-caudal) projections lateral to the sagittal stratum (green) in the posterior temporal lobe corresponding to the vertical portion of the AF were delineated<sup>(30)</sup>.

### Inferior fronto-occipital fasciculus

For the IFOF (Figure 2), the seed ROI was placed by delineating the green projections in the entire anterior part of the frontal lobe on the axial and/or coronal slices. The target ROI was placed by delineating the green projections in the entire posterior part of the occipital lobe up until the posterior crossing area between the genu of the corpus callosum (red) and the posterior part of the sagittal striatum (green) on the axial and/or coronal slices<sup>(30)</sup>.

### Uncinate fasciculus

For the UF (Figure 3), the seed ROI was placed in the green projections in the temporal pole/ entire anterior part of the temporal lobe on the coronal slice. The target ROI was

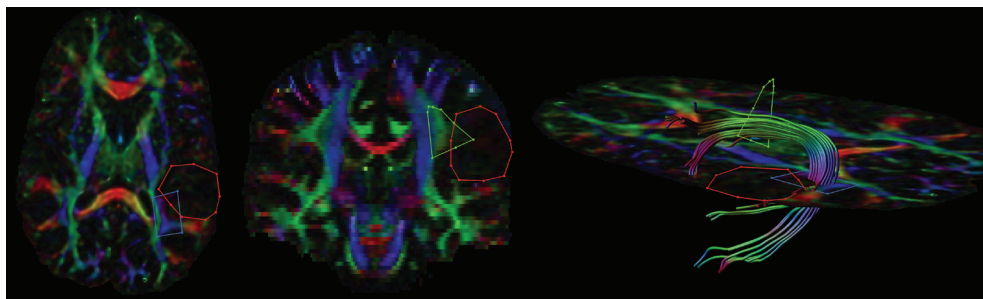
placed by delineating the green projections in the inferior part of the frontal lobe on the coronal slice<sup>(30)</sup>.

### Neuropsychological assessment

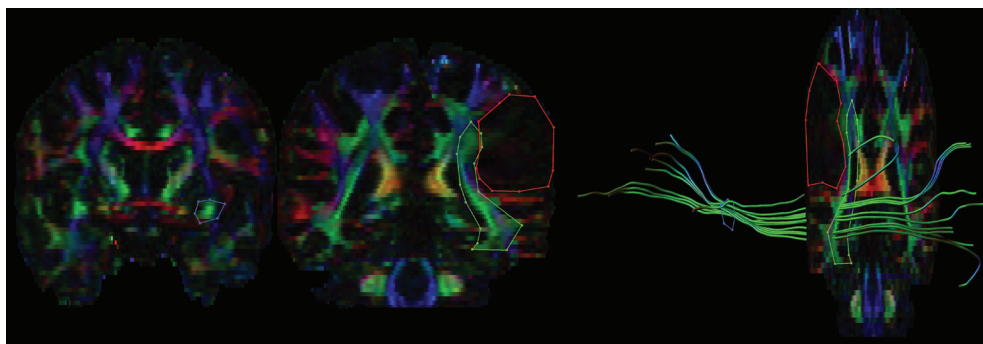
All patients were assessed once with a comprehensive NP test battery after initial diagnosis (mean= 9 days; SD= 20) and before surgery (mean= 44 days; SD= 30) by a clinical linguist (blinded for tractography results). As shown in Table 2<sup>(12,14,18,20,27,33)</sup>, the performance of the patients was examined within three main cognitive domains (language, memory and attention/executive functions). This combination of NP tests has shown to be sensitive to detect cognitive deficits and has been validated in glioma patients in earlier studies<sup>(24,26,31)</sup>. All test-scores of the patients were transformed into z-values to compare the performance of patients to a normative group and to facilitate comparisons between tests.

### Statistical Analysis

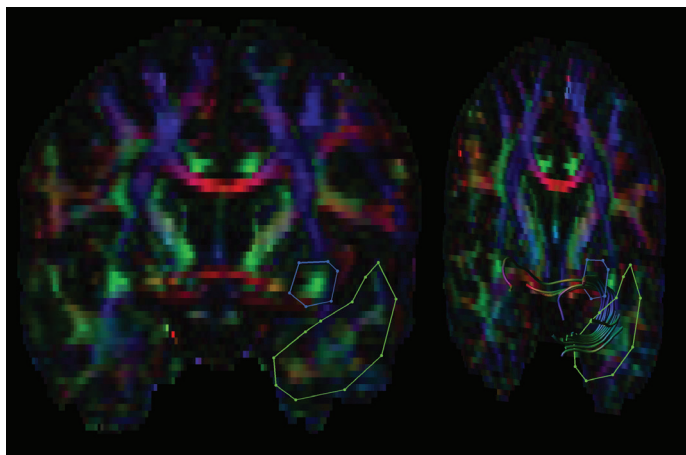
Statistical analyses were performed with the SPSS statistical software (SPSS version 21.0, SPSS Inc. Chicago, IL, USA). We determined whether patients' mean scores by cognitive domain and by test differed from the normative group, using either a one-sample t-test with 0 (the mean score of the normal group) as test value or the Wilcoxon Signed Rank Test. Post-hoc, mean FA/MD of the three WM tracts and three cognitive domains were compared between histopathological established LGG and HGG by using a one-way ANOVA. First the relationship between the three main cognitive domains (language, memory and attention/executive functions) of the NP and FA/MD values of three WM tracts (AF, IFOF, UF) was analyzed in linear regression models which included all three WM tracts, with age and histopathological grade as regressors of no interest. The analyses were done separately for each main cognitive domain and each DTI metric (FA, MD). Bonferroni correction was used to adjust for multiple testing on main cognitive domain level (6 tests): a p value of  $\leq 0.008$  was considered to be statistically significant. Then, further analyses were done on subtest level, only within the cognitive domain(s) that were significantly correlated with WM tract measure(s). Bonferroni correction was not used for these analyses: a p value of  $<0.05$  was considered to be significant.



**Figure 1.** The first ROI is placed on the green triangular shaped projections lateral to the blue projections of the cortical spinal tract in the posterior parietal lobe. The second ROI is placed on the blue projections laterally to the sagittal stratum (green). The tumor is delineated in red. The 3D-reconstruction of the AF is shown at the most right figure.



**Figure 2.** The first ROI is placed on the green projections in the anterior part of the frontal lobe. The second ROI is placed on the green projections in the posterior part of the occipital lobe. The tumor is delineated in red. The 3D-reconstruction of the IFOF is shown at the most right figure.



**Figure 3.** The first ROI is placed on the green projections in the anterior part of the temporal lobe. The second ROI is placed on the green projections in the inferior part of the frontal lobe. The tumor is delineated in red. The 3D-reconstruction of the UF is shown at the most right figure.

**Table 2.** Neuropsychological test protocol with z-scores and p-values per cognitive domain (standard deviations).

Neuropsychological test	N <sup>a</sup>	Cognitive Abilities	Description	Mean z-score (SD)	P-value
<b>Language domain</b>				-0.78 (0.98)	<0.0001
Aachener Aphasia Token Test (AAT TT) (14)	75	Language comprehension; severity of language disorder	Pointing to and manipulating geometric forms on verbal commands	-0.40 (1.97)	0.740
Aachener Aphasia repetition (AAT rep) (14)	72	Repetition	Repeating phonemes, words and sentences	-0.11 (0.81)	0.013
Boston Naming Test (BNT) (15)	75	Naming (word finding)	Naming 60 pictures, presented in order of word frequency and word difficulty	-1.52 (1.72)	<0.0001
Category fluency (CAT) (16)	74	Flexibility of verbal semantic thought processing; working memory	Producing words of a given category (animals and professions) within a limited time span	-1.04 (0.84)	<0.0001
Letter fluency (LET) (17)	73	Flexibility of verbal phonological thought processing; working memory	Producing words beginning with a given letter (D,A,T) within a limited time span	-0.89 (1.19)	<0.0001
<b>Memory domain</b>				-1.49 (1.35)	<0.0001
15 Words test (WT) imprinting	62	Verbal learning; immediate and delayed recall	Learning a list of 15 words, with 6 recall trials; 5 immediate and 1 delayed,	-1.82 (1.43)	<0.0001
15 Words test (WT) recall (18)	62			-1.22 (1.36)	<0.0001
<b>Attention and executive functions</b>				-0.57 (0.93)	<0.0001
Trail Making A	65	Visuomotor speed, attention	Connecting numbers placed randomly in ascending order as rapidly as possible	-0.60 (1.14)	<0.0001
Trail Making B	63	Mental flexibility, divided attention		-0.37 (1.28)	0.027
Trail Making BA (19)	62			-0.12 (1.23)	0.459
Stroop I	53	Mental speed; selective attention	Reading colour words, naming colours and naming colours of printed words, denoting another colour	-1.09 (1.39)	<0.0001
Stroop II	53			-1.12 (1.35)	<0.0001
Stroop III	51			-0.64 (1.06)	<0.0001
Stroop interference	48			-0.31 (1.09)	0.056

<sup>a</sup> Number of patients assessed before surgery. In some cases, the full protocol could not be applied to all patients due to several reasons. Priority was given to tests that were most relevant to the preparation of the operative procedure.

## Results

### DTI tractography

Successful tractography was performed in 68 patients of the AF (88%), in 76 of the IFOF (99%), and in 74 of the UF (96%). In total, 13 (17%) WM tracts could not be tracked due to very large tumor volume causing extensive brain shift. These WM tracts were not included in the analysis of association with NP tests. Mean FA was 0.456 (SD = 0.059) in the AF, 0.474 (SD = 0.056) in the IFOF, and 0.395 (SD = 0.094) in the UF. Mean FA and MD of the three WM tracts was not significantly different ( $p > 0.05$ ) between LGG (N = 47) and high-grade glioma (HGG, N = 30).

### Patients' performance on NP

Patients had impairments in all cognitive domains (language, memory and attention/executive functions) compared to the normative group ( $p < 0.05$ ), except for three (sub) tests (Table 2). These were in the language domain (AAT Token Test) and in the attention/executive functions (TMTBA and Stroop interference) ( $p > 0.05$ ). Post-hoc analyses showed that language impairments were significantly worse in patients with histopathological determined high grade than those with low grade tumors ( $p = 0.025$ ). Memory and attention/executive functions were not significantly different between these groups ( $p > 0.05$ ).

### Relationship between NP and WM tracts

The relationship between the three main cognitive domains and the FA and MD values of the three WM tracts is presented in Table 3. Significant correlations were found between the AF (FA only) and the language domain ( $\beta = 0.44$ ;  $R = 0.40$ ;  $p = 0.003$ ) and between the IFOF (FA only) and the memory domain ( $\beta = -0.48$ ;  $R = 0.44$ ;  $p = 0.006$ ) and the attention/executive function domain ( $\beta = -0.49$ ;  $R = 0.45$ ;  $p = 0.008$ ). Additional correlations that approached significance were seen between both the FA and MD of the AF, IFOF and memory and attention/executive functions domain ( $0.008 < p < 0.05$ ). No significant correlations were found between the UF and any of the three cognitive domains assessed with the NP ( $p > 0.05$ ).

Within the language domain, a strong significant correlation was found between the FA in the AF and the "Aachener Aphasia Repetition Test (AAT rep)" ( $\beta = 0.59$ ;  $R = 0.53$ ;  $p < 0.0001$ ). The IFOF was significantly correlated with a subtest of the memory domain for verbal memory: "15 Words Test Imprinting" ( $\beta = -0.55$ ;  $R = 0.48$ ;  $p = 0.002$ ) and with a subtest for selective attention within the attention/executive function domain: "STROOP I" ( $\beta = -0.62$ ;  $R = 0.50$ ;  $p = 0.006$ ) (Table 4).



**Table 3.** Relationship between 3 neuropsychological domains (language, memory, attention/executive functions) and FA/MD values of 3 white matter tracts (AF, IFOF, UF).

	Arcuate Fasciculus		Inferior fronto-occipital fasciculus		Uncinate fasciculus	
Domain	FA	MD	FA	MD	FA	MD
LANG n=65	$\beta = 0.44$ P = 0.003 <sup>b</sup> R=0.40	$\beta = -0.31$ P = 0.380 R=0.28	$\beta = -0.30$ P = 0.057 R=0.40	$\beta = 0.12$ P = 0.499 R=0.28	$\beta = 0.06$ P = 0.665 R=0.40	$\beta = 0.05$ P = 0.758 R=0.28
MEM N=54	$\beta = 0.34$ P = 0.029 <sup>a</sup> R=0.44	$\beta = -0.21$ P = 0.172 R=0.37	$\beta = -0.48$ P = 0.006 <sup>b</sup> R=0.44	$\beta = 0.45$ P = 0.019 <sup>a</sup> R=0.37	$\beta = 0.32$ P = 0.055 R=0.44	$\beta = -0.27$ P = 0.128 R=0.37
ATT/EX N = 55	$\beta = 0.39$ P = 0.014 <sup>a</sup> R=0.45	$\beta = -0.33$ P = 0.036 <sup>a</sup> R=0.32	$\beta = -0.49$ P = 0.008 <sup>b</sup> R=0.45	$\beta = 0.04$ P = 0.847 R=0.32	$\beta = 0.17$ P = 0.339 R=0.45	$\beta = 0.09$ P = 0.656 R=0.32

Linear regression models with NP as a dependent factor, FA/MD and age as independent factors.  $\beta$  = standardized coefficient R = correlation coefficient. LANG = language, MEM = memory, ATT/EX = attention/executive function, FA = fractional anisotropy, MD = mean diffusivity <sup>a</sup> indicates statistical significance at level of  $p < 0.05$  <sup>b</sup> indicates statistical significance at level of  $p \leq 0.008$ , see table 4 for subtasks associations

**Table 4.** Association between the significant subtasks of the significant cognitive domains and fractional anisotropy in the arcuate fasciculus and inferior.

Domain	Subtask	White matter Tract
Language	AAT REP	AF, $\beta = 0.59$ P = 0.000 R=0.53
Memory	15 WT IMPR	IFOF, $\beta = -0.55$ P = 0.002 R=0.48
Attention and executive functions	STROOP I	IFOF, $\beta = -0.62$ P = 0.006 R=0.50

SD standard deviation, IQR Inter Quartile Range, CE contrast enhancement, FLAIR Fluid-attenuated inversion recovery.

Linear regression models with NP as a dependent factor, FA, grade and age as independent factors.  $\beta$  = standardized coefficient. R = correlation coefficient. AAT REP = Aachenner Aphasia Repetition Test, 15 WT IMPR = 15 Words Test Imprinting AF = arcuate fasciculus, IFOF = inferior fronto-occipital fasciculus

## Discussion

We performed a large-scale quantitative analysis of language WM tract microarchitecture and cognitive performance in patients with presumed LGG. Patients had deficits in all assessed cognitive domains, i.e. language, memory and attention/executive functions. A significant correlation was found between FA in the AF and the language domain, specifically with a repetition test, which is associated with phonological abilities. The FA in the IFOF was significantly correlated with deficits in verbal learning (imprinting) and selective attention. In this cohort of patients with tumors that radiologically appear to be low grade (i.e. presumed LGG), language impairments were significantly worse in patients with tumors that turned out to be high grade upon histopathological examination.

### DTI measures within the language WM tracts

We found that lower FA, but not MD, was significantly correlated with language and verbal memory deficits in patients with presumed LGG. FA is a measure of directionality of molecular motion classically known to be sensitive to microstructural changes, but not specific to the type of changes. MD is a measure of magnitude of diffusion and is sensitive to cellularity, edema and necrosis <sup>2</sup>. Several tumor associated mechanisms are described that influence FA and MD. Tumor infiltration in WM tracts as well as edema may cause a decrease in FA and increase in MD, while compression of WM tracts due to tumor causes an increase in FA and decrease in MD <sup>15</sup>. Lee et al showed that purely vasogenic edema, composed purely of extracellular water (e.g. with meningiomas) causes a more relative increase in MD than a decrease in FA. On the other hand, tumor infiltrated edema - infiltration of tumor (e.g. glioma) cells in WM tracts - causes a more relative decrease in FA than an increase in MD <sup>16</sup>. We can therefore speculate that our findings may reflect tumor infiltration into the white matter tracts.

### Arcuate fasciculus and language deficits

We demonstrated an association between microarchitectural changes of the AF and impaired language repetition abilities. The AF, also known as the dorsal pathway in the dual stream model of Hickok & Poeppel <sup>13</sup>, is associated with mapping sound to articulatory-based representations (e.g. phonology), which explains the functional association between microstructural changes and phonological deficits observed here. Our findings also support recent results from Sierpowska et al. <sup>29</sup>. They reported that monitoring (non) word repetition is relevant near the AF during electro(sub)cortical stimulation for preservation of language production, in particular phonological performance <sup>25,29</sup>.

Deficits in language repetition in combination with relatively intact comprehension, as observed in our patient group, is “classically” known as conduction aphasia in the stroke literature. Several studies in patients with different etiologies have demonstrated a link between this specific aphasia type and damage to the AF (e.g. tumor infiltration, WM

tract compression)<sup>4</sup>. In addition, damage to AF may be predictive for persistent language deficits<sup>6,22</sup> at test level, but also in relation to the quality of communication, underlining the importance of a repetition task.

### **Inferior fronto-occipital fasciculus and cognition**

Another important pathway, apart from AF, that is reported to be related to language is the IFOF, which is part of the so-called ventral stream according to Hickok & Poeppel<sup>13</sup>. The ventral stream (IFOF) is involved in mapping sound on meaning (e.g. semantics, comprehension). In our study, the participants had no comprehension deficits, as measured with the Token Test, when compared with healthy people, and it is therefore not surprising that no association was found between DTI measures within the IFOF and the language domain. Within the semantic level, there were impairments in category fluency, but the multidimensional background of this test, i.e. language (lexical retrieval), semantic memory and in part also attention/executive functioning, may be responsible for the lack of a correlation. While it is possible that there were indeed no (clear) semantic deficits in our patient population, this finding may also indicate a limitation in our NP test procedure in that the applied tests for language functioning were not equivalent to the main linguistic components: semantics, syntax and phonology. In contrast with phonology, semantics and syntax were more globally examined by means of the Token Test. We have therefore developed a more elaborate test-battery for language functioning in glioma patients: the Dutch Linguistic Intraoperative Protocol (DuLIP)<sup>7</sup>. This is currently in the phase of evaluation.

Besides language deficits, it is suggested that damage to this WM tract can also cause other cognitive deficits. According to the model proposed by Duffau<sup>8</sup>, the IFOF could be relevant for attention and/or (working) memory. Our study showed a correlation between FA in the IFOF and both imprinting and attention deficits, which provides additional support for previous suggestions on the involvement of the IFOF in memory.

The lack of clear cognitive deficits in other domains could be explained by neural plasticity, i.e. functional reorganization. Tumors in all our patients were presumed to be LGG (non-to-mild contrast enhancing), and in more than half of these patient's low-grade tumor was confirmed upon histopathological examination. LGG is typically associated with relatively slow tumor growth rate facilitating neural plasticity. As a result, functional reorganization could have taken place, which would lead to less severe deficits than expected from the degree of tract infiltration. More sensitive tasks to detect mild cognitive deficits in LGG patients would therefore be useful.

### **Tumor grade**

Of note in this context is the finding that a large proportion (41.6%) of non-to-mild enhancing glioma in this study turned out to be HGG upon histopathological examination. This could have influenced our findings if compared to a purely LGG group. However,

whether and how LGG influences FA/MD values in perilesional WM tracts differently from HGG is still under debate<sup>19,23</sup>. In this study, language impairments were significantly worse in histopathological established HGG than in LGG, while there was no significant difference in FA or MD values of the three WM tracts between these two groups. The former is consistent with the more aggressive nature of these tumors, the latter with their radiological characteristics. We can speculate that the higher grade of these tumors allows for less plasticity and hence worse deficits.

### **Limitations**

There were some limitations to our study. Deterministic tractography was used to identify three WM tracts per patient by placing two ROIs per WM tract. It is important to note that the procedure of manually anatomical seed ROI placement is subject to variability within and between raters. Subjectivity depends on the location of ROI placement and the WM of interest, with a higher variability of tracking in cortical areas and higher variability within specific tracts such as the inferior longitudinal fasciculus<sup>21,28</sup>. This is even more relevant in the context of brain tumors, as Schonberg et al. showed that when WM tracts are displaced by excessive edema or shift due to tumor compression, the variability and subjectivity of tractography increases significantly when compared with the contralateral hemisphere<sup>28</sup>. We attempted to keep the inter-subject variability low and success rate high, by using a single, well-trained rater who placed ROIs systematically on clearly identifiable anatomical landmarks on DTI maps using published approaches<sup>30</sup> and who visually inspected each identified WM tract in three dimensions. Additionally, the exclusion of WM tracts that could not be tracked in this analysis could have introduced some selection bias, since these patients potentially have more or more severe cognitive deficits. Furthermore, one could argue that the diffusion imaging acquisition is not fully state-of-the-art in the context of neuroscientific research, which now includes multiple b-values and many more gradient directions. This study was performed within the constraints of a routine clinical context, in which longer scan durations are not feasible. This may have limited our sensitivity to more subtle changes or associations, which means our negative findings need to be interpreted with care. A further reason why to interpret findings in DTI studies with care is due to the possibility that the difference in magnet strength (1.5 T vs 3.0T) can cause a difference in FA/MD value in the WM tracts<sup>11</sup>. This subsequently is likely to cause reduced strengths of associations between FA/MD values and cognitive outcome. However, we did not find a difference in FA/MD values between the two magnet strengths in this study and we can fairly confidently state that the associations we did find were valid and strong findings.

Finally, due to multiple testing, a stringent significance level was maintained. As a result, there is the risk of false-negative findings and relevant correlations could have been obscured. On the other hand, the risk of false-positive findings is low and correlations that did pass the stringent significance level can be considered clearly positive.

## Conclusion

This clinical DTI study shows that pre-operative language deficits in repetition of speech, verbal learning and attention deficits are associated with changes in the microarchitecture of respectively the AF and the IFOF in patients with presumed LGG. This indicates that especially repetition tasks during surgery are very important and should be implemented in the NP as a standard. Verbal learning is difficult to monitor during surgery, which highlights the need for a careful pre-operative assessment of the IFOF with DTI, especially in patients with decreased performance on the 15WT. We emphasize that, especially in patients with deficits in speech repetition or verbal learning, performing extensive tumor resection will need to be balanced carefully against preserving infiltrated WM tracts (AF, IFOF). Further investigation is needed to assess the predictive value of pre-operative FA of WM tracts, tumor genetic profile, and extent of tumor resection on post-operative cognitive outcome.



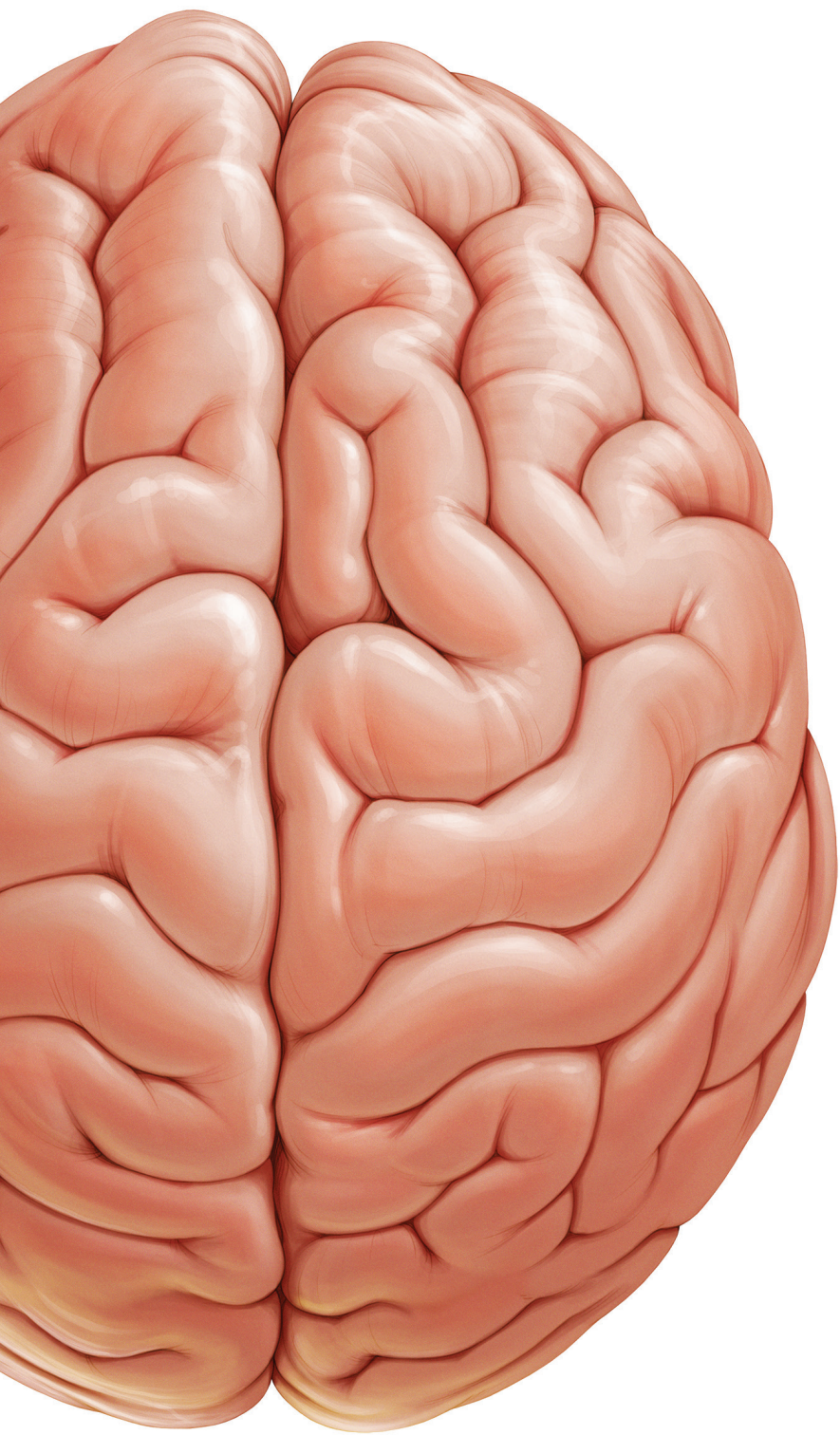
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# PART II

## Image-guided glioblastoma surgery



# Chapter 6

## Intraoperative ultrasound guided surgery and the extent of glioblastoma resection: a randomized, controlled trial

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Iain Haitsma, Joost Schouten, A.J.P.E. Vincent

*Submitted*







# Chapter 7

## Clinical feasibility of a wearable mixed reality device in neurosurgery

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## **Abstract**

### **Background**

Neuronavigation systems are routinely used during neurosurgical procedures. Currently, new imaging technologies are emerging such as virtual, augmented and mixed reality. With mixed reality devices, the user can analyze and interact with the real environment using virtual objects. The aim of this prospective pilot study was to offer a proof of concept by testing the clinical feasibility and accuracy of a wearable mixed reality device (Hololens) for pre-operative neurosurgical planning.

### **Methods**

In patients with an indication for brain tumor surgery, pre-operative planning of tumor localization with the Hololens was compared with standard neuronavigation in the operating room. Magnetic resonance imaging based 3D holograms of the patient's head and tumor were created and projected on the physical patient's head using the Hololens. The 2D projection of the tumor borders as perceived by the neurosurgeon on the skin of the patient's head was outlined both with the Hololens and neuronavigation. Accuracy of the Hololens localization was assessed using neuronavigation as the gold standard.

### **Results**

Twenty-five patients were included in this study. Holograms were successfully created in all cases. In nine patients, tumor localization with the Hololens did not differ from the standard neuronavigation system and the overall median difference was 0.4 cm (IQR 0-0.8).

### **Conclusion**

This prospective clinical study offers a proof of concept of the clinical feasibility of the Hololens for brain tumor surgery planning in the operating room, with quantitative outcome measures. Further development is needed to improve the accuracy of this wearable mixed reality device.

## Introduction

In the early 1990s the first commercially available neuronavigation system was introduced.<sup>(1)</sup> Image guided navigation technologies have undergone several developments since then which have led to important improvements during both pre- and intra-operative neurosurgical procedures. Although limited evidence of an improvement of survival or quality of life of patients with neuronavigation guidance is available, neuronavigation is a highly practical and time efficient tool for pre- and intra-operative target localization.<sup>(2,3)</sup> Therefore, now almost 30 years later, the neuronavigation system has found its place in the operating room as a standard tool in cranial neurosurgery. Neuronavigation consists of several important applications, one of which is the pre-operative localization of the target lesion on the outer surface of a patient's head. However, this system has a considerable setup time due to a coordinate-based registration procedure. Additionally, the surgeon needs to translate these coordinates from a monitor to the patient's head for an accurate target localization.

Currently, we are seeing newly emerging imaging technologies which have a potential medical applicability by creating virtual objects in the real, physical world, and the ability of interaction between these objects and the real world. Virtual reality (VR), in which a real environment is replaced by a virtual one, and augmented reality (AR), in which virtual objects are overlaid on a real, physical environment, are such innovative technologies.<sup>(4,5)</sup> These new technologies are potentially of great benefit to neurosurgeons for complex surgical procedures. Meola et al. systematically reviewed AR studies within the field of neurosurgery and they concluded that AR has a potential benefit to improve current neuronavigation systems, but that prospective and clinical application studies are very limited.<sup>(6)</sup>

In contrast to VR and AR, mixed reality can be used to analyze and interact with the real environment by placing a virtual object over the physical environment. Our hypothesis is that mixed reality can be used for pre-operative planning and localization of the tumor by placing 3D hologram reconstructions of pre-operatively acquired brain MRI or CT scans over the physical operating field. To the best of our knowledge, a wearable mixed reality device has as yet not been tested within the field of (oncological) neurosurgery in a prospective clinical study. The aim of this study was to test the clinical applicability and accuracy of a wearable mixed reality device for pre-operative neurosurgical tumor localization and planning compared with standard neuronavigation.

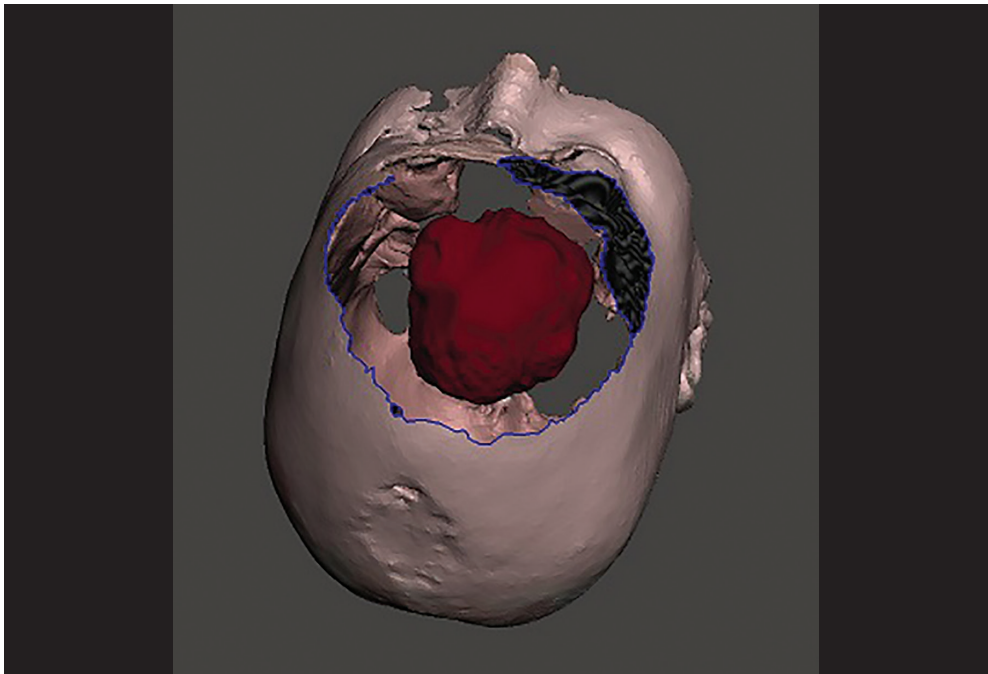
## Methods

### Study participants

Patients with the age of 18 years and older, referred for neurosurgery of a newly diagnosed contrast enhancing intra- or extra-axial lesion as seen on a pre-operative T1 with contrast MRI scan, suspected for brain tumor, between December 2016 and December 2017 at the department of neurosurgery, Erasmus MC were eligible for this study. This study was approved by the IRB. Obtaining written informed consent was waived by the IRB, because participants were not subjected to any specific study procedures nor were they required to follow rules of behavior, and no additional risk was introduced by the study.

### Hologram creation

Tumor volumes were semi-automatically segmented on high-resolution 3D contrast enhanced T1 weighted MRI images using a free online toolkit (ITK-SNAP; [www.itksnap.org](http://www.itksnap.org)) and 3D objects (Figure 1) were reconstructed using free online software (Meshmixer; [www.meshmixer.com](http://www.meshmixer.com)). Then, 3D objects were sent online to the Hologlens using a commercially available application (VertoStudio; [www.vertostudio.com](http://www.vertostudio.com)). The Microsoft Hologlens is a commercially available, wearable computer integrated mixed reality device ([www.microsoft.com/microsoft-hologlens](http://www.microsoft.com/microsoft-hologlens)).



**Figure 1.** 3D reconstruction of the head of the patient with the edges of the virtual craniotomy (blue) and the tumor (red). This model is converted to a hologram and send to the mixed reality device.

### Operative planning with the Hololens

From tumor segmentations, tumor volumes (cm<sup>3</sup>) were measured using ITK-SNAP. Tumors were categorized as superficial (distance of 0 cm) or deep tumors (distance > 0 cm), as measured from the tumor border closest to brain surface to brain surface. Additionally, based on their localization, tumors were categorized by hemisphere (right vs. left) and lobe (frontal, parietal, temporal and occipital). Planning time for both the Hololens as the standard navigation system were measured from time to system setup to the final tumor outline on the patient's head. In the operating room, skin surface registration was performed manually by merging the 3D holograms of the patient's head and tumor with the physical head of the patient. Then, the 2D projection of the holographic tumor borders and the center of the tumor were marked on the skin of the patient's head as perceived by the neurosurgeon wearing the Hololens (Figure 2 and Figure 3). The center and outlines of the tumor were then marked again on the skin of the patient's head using the standard neuronavigation system (BrainLab, Feldkirchen, Germany). The maximal extension of the borders of the tumors were marked in 4 directions: anterior, posterior, medial and lateral. The exact center of the tumor projection on the skin was determined by the crossing of the anterior-posterior line and the medial-lateral line. The same was done with the standard neuronavigation system. As an outcome for accuracy in tumor localization, the maximum distance (cm) between the center of the tumor determined with the Hololens versus with neuronavigation (as the gold standard) was measured on the patient's skin. Eventually, skin incisions, craniotomy and surgery were performed based on tumor localization using the standard neuronavigation system. All measurements were performed by authors with prior Hololens experience (F.I and/or A.V). Cases were excluded from this study, if both of the authors could not attend surgery.



**Figure 2.** The head of the patient as seen through the Hololens. The 3D hologram is merged with the physical head of the patient. The tumor (red) is outlined first with the Hololens (light blue dots) and then with the navigation system (black dots). The two contours are in line with each other.



**Figure 3.** Neurosurgeon with the Hololens in the operating room.

### **Qualitative assessment**

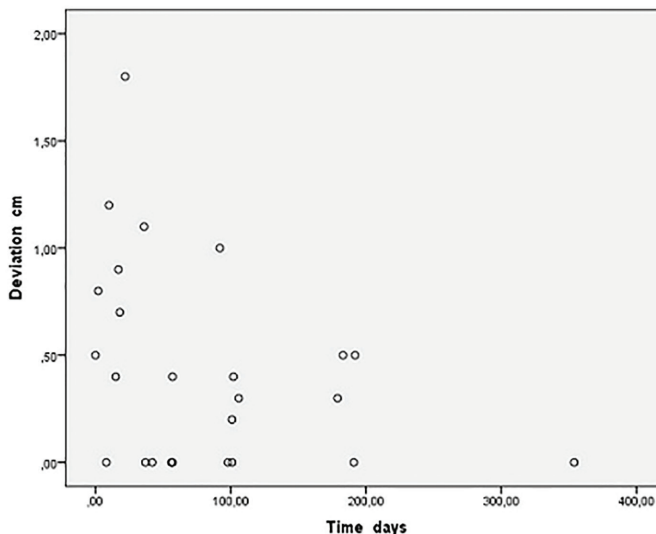
To obtain an impression of the alignment of the Hololens tumor projection in full, the holographic tumor borders of a meningioma were compared qualitatively with the true meningioma borders as seen on the brain surface, after opening the dura, in one patient. This assessment was also compared with the pre-operative tumor outlines on the skin.

### **Statistical Analysis**

Statistical analyses were performed with the SPSS statistical software (SPSS version 21.0, SPSS Inc. Chicago, IL, USA). A one-sample Wilcoxon signed rank test was performed to test whether there was a significant deviation between Hololens and neuronavigation. Differences in deviation between groups of the various tumor locations between the Hololens and neuronavigation system were tested with a one-way ANOVA test. The difference in deviation between superficial and deep tumors and between the first and second half of the study, as well as the difference in pre-operative planning time between Hololens and standard neuronavigation were tested with a t-test. A p value of  $<0.05$  was considered to be statistically significant.

## Results

Twenty-five patients were included in this study with a mean age of 57 years (range 22–80). Tumors had a median volume of 34.8 cm<sup>3</sup> (IQR 5.8 – 58.2) and 17 of 25 (68%) tumors reached the brain surface. Patient and tumor characteristics are further presented in Table 1. Holograms were successfully created in all of the patients. Mean pre-operative planning time with the HoloLens (mean 5 min 20 sec, SD 1 min 20 sec) was longer, when compared with the standard neuronavigation system (mean 4 min 25 sec, SD 1 min 20 sec,  $p < 0.001$ ). In nine patients (36%) tumor localization with the HoloLens did not differ from that of the standard neuronavigation system. Overall, there was significant deviation between the HoloLens and neuronavigation ( $p < 0.0001$ ), with a median deviation of 0.4 cm (IQR 0–0.8). There was no statistically significant difference in deviation between tumor location in the left (median = 0.3 cm, IQR 0–0.5) and right hemisphere (median = 0.5 cm, IQR 0–1.1,  $p = 0.17$ ) or between the frontal, parietal, temporal and occipital lobes ( $p = 0.74$ ). Additionally, no significantly different deviation was found between superficial (median = 0.4 cm, IQR 0–0.8) and deep tumors (median = 0.25 cm, IQR 0–0.8,  $p = 0.65$ ). There was a trend towards more accurately localizing tumors towards the end of the study (Figure 4), with a median deviation of 0.6 cm (IQR 0–1.1) during the first half ( $n = 12$ ) of the study and a median deviation of 0.3 cm (IQR 0–0.5) during the second half ( $n = 13$ ) of the study ( $p = 0.07$ ). The qualitative assessment method showed that after opening the dura, the tumor as seen on the brain surface, was within the borders of the holographic tumor and also within the contour as marked on the patient's head using the HoloLens.



**Figure 4.** A scatterplot of deviation (cm) on the y-axis over time (days) on the x-axis, with  $t = 0$  inclusion of first patient.

**Table 1.** Patient and tumor characteristics with deviation in tumor localization with Hololens, compared with neuronavigation system.

Age	Sex	Tumor localization	Tumor volume (cm3)	Tumor depth *	Pathology	Time pre-operative planning Hololens   navigation (min)	Deviation Hololens from navigation (cm)
71	Female	Right parietal	5.2	2.3	Glioblastoma	6   4	0.5
80	Male	Right temporal	9.4	0	Glioblastoma	5   3	0.8
38	Male	Left temporal	72.5	0	Meningioma	3   4	0
48	Female	Right frontal	29.1	0	Meningioma	7   5	1.2
67	Male	Left frontal	2.0	0	Glioblastoma	3   5	0.4
64	Male	Left temporal	94.4	1.2	Meningioma	5   4	0.9
54	Female	Right occipital	34.8	0	Metastasis	6   8	0.7
73	Male	Right frontal	1.8	0	Lymphoma	4   4	1.8
57	Male	Right temporal	6.4	24.0	Glioblastoma	5   4	1.1
53	Female	Left parietal	87.5	0	Glioblastoma	7   8	0
65	Male	Left temporal	35.1	1.3	Glioblastoma	7   4	0
70	Female	Left frontal	54.5	0	Metastasis	5   4	0
54	Male	Right frontal	45.5	0	Glioblastoma	5   3	0.4
73	Female	Right parietal	44.3	0	Glioblastoma	4   3	0
77	Female	Left frontal	32.9	0	Glioblastoma	3   3	1.0
63	Female	Left frontal	35.9	1.4	Glioblastoma	6   4	0
22	Female	Left frontal	36.2	0.5	Astrocytoma	5   5	0.2
63	Female	Right temporal	11.7	0.8	Meningioma	4   5	0
62	Female	Left frontal	61.9	0	Glioblastoma	5   5	0.4
28	Female	Left frontal	4.2	0.6	Astrocytoma	6   3	0.3
51	Male	Left temporal	114.7	0	Metastasis	8   4	0.3
24	Female	Left frontal	0.2	0	Hemangioblastoma	5   5	0.5
74	Male	Right frontal	29.3	0	Meningioma	6   5	0
54	Female	Left occipital	3.6	0	Glioblastoma	5   6	0.5
37	Female	Right frontal	161.0	0	Meningioma	7   4	0

\* Zero indicates that tumor reached brain surface.



## Discussion

This prospective clinical study offers a proof of concept of the clinical feasibility of a wearable mixed reality device for pre-operative neurosurgical planning using 3D hologram reconstructions of brain tumors with quantitative outcome measures. This study shows that there was a median deviation of 0.4 cm in tumor localization, when the HoloLens was compared with the standard neuronavigation system. In nine out of 25 patients the localization did not differ between both systems. Additionally, the measurement strategy used in this study, focusing on the tumor center, was qualitatively assessed for full tumor alignment and showed that the full tumor alignment on the patient's head using the HoloLens corresponds with the true tumor borders as seen on the brain surface.

Surgeons experienced benefits in terms of preservation of attention and focus on the patient, improved ergonomics, and an improved understanding of tumor-brain/skull relationship due to a direct 3D holographic representation. To the best of our knowledge, there have been no studies on wearable mixed reality devices with 3D virtual projections for brain tumor surgery to this date. We emphasize that spatial mapping and recognition of the physical environment is a crucial difference between augmented and mixed reality. Mixed reality with the HoloLens provided an instant visualization of tumors by using 3D holograms. Due to a built-in computer in the HoloLens, other devices such as camera, monitor or excessive technical preparation at the operating room were not necessary. In contrast to current neuronavigation systems, the HoloLens subjectively improved ergonomics during surgical planning, because neurosurgeons could keep their focus on patient's head, instead of having to look back and forth between patient and navigation screen. Importantly, during this study, there was a learning curve for neurosurgeons in using this wearable mixed reality device in localizing and marking tumor border on the patient's head as evidenced by a trend towards more accurately localizing tumors towards the end of the study.

Within the field of neurosurgery, VR has played a limited role in pre- and intra-operative application due to the fact that the neurosurgeon is isolated in a virtual world, while being in the operating room where situational awareness is crucial. However, for extra-operative pre-surgical planning and education, VR devices are useful to improve the understanding of both individual anatomy of patients<sup>7</sup>, anatomical education<sup>8</sup> and neurosurgical training<sup>5</sup>. Meola et al. reviewed 18 articles on AR in neurosurgery<sup>6</sup> published between 1996 and 2015. Microscopes<sup>9-16</sup> and monitors or tablets<sup>17-24</sup> were the most commonly used AR devices, which were all 2D visualizations of virtual objects such as aneurysms and brain tumors. Monitors or tablets could be practical AR devices to use for pre-operative surgical planning. However, with these devices, an in-physical-space 3D visualization of virtual objects of complex anatomical structures is limited. Additionally, microscopes offer some 3D experience of virtual objects such as tumor, fiber tracts and



vessels through the oculars. However, the microscope is commonly used after trepanation in the intra-operative phase, instead of a pre-operative planning device.

Recently, Yoon et al. reviewed the literature between 1995 and 2017 extensively on several augmented reality devices within all surgical fields.<sup>25</sup> Within the field of neurosurgery, they identified 10 studies, of which 5 performed surgery in a live setting and 5 performed simulated surgeries. There were no studies that had used the HoloLens. The authors found that wearable augmented reality devices improved attention, focus and ergonomics compared to standard navigation systems in which the surgeon has to shift repeatedly from 2D images on a screen to the patient. The authors also state that the majority of the studies had not assessed any quantitative outcome measure. The experience and results in our study concerning the preservation of attention and focus by the surgeon on the patient, the improved 3D anatomical evaluation, and improved ergonomics are in line with the conclusions of this systematic review.

This study provides a proof of concept that the HoloLens has potential for operative planning of brain tumor surgery with quantitative outcome measures. However, we think that there are two important issues that need further development to improve the accuracy of the HoloLens. Due to the manual skin surface registration, the accuracy was probably decreased and registration time longer as compared to the standard neuronavigation. The HoloLens deviated with a median of 0.4 cm in localizing the tumor and prolonged median registration time with 45 seconds. In the setting of operative planning, this deviation is probably acceptable, it is however too large for intra-operative usage. Currently, we are working on a coordination-based skin surface registration system which is expected to improve spatial accuracy and decrease registration time. Additionally, there was an issue with respect to the point of view when marking tumor border on the skin of the patient's head with the HoloLens, when compared with standard neuronavigation. Since the holographic head is 3D and transparent, the tumor can be seen and marked from different angles. Any angle that is not exactly perpendicular to the head and tumor, will result in a deviation of localizing the lesion when compared with standard neuronavigation.

A limitation of this study is that a non-validated method was used in this study to outline the tumor. This method is standard practice with the use of the neuronavigation system in our neurosurgical center, and we therefore chose to use this method also in context of this study for the HoloLens. Another limitation of this study is that patients were not consecutively included in this study, which could have introduced selection bias based on case complexity. Most of the tumors were superficially located. However, we did have some complex cases with tumors in the basal ganglia or skull base. We did not find any significant difference in accuracy between superficial and deep tumors. In cases with skull base tumors, surgeons reported a better understanding of tumor-brain/skull base relationship.

Further research is needed to test the applicability and accuracy of mixed reality devices in several other neurosurgical fields such as pre and per-operative planning of

neurosurgery by visualizing neuroanatomical structures (such as more complex tumors, aneurysms, vascular malformations, skull base tumors, and disc herniations). Additional options of this technology should be explored, which includes overlaying patient (history, vital statistics) and imaging data (MRI and/or CT scan) directly over the field of view of the surgeon, which could further improve interpretation, attention and focus of the surgeon during surgery.

## **Conclusion**

This prospective clinical study offers a proof of concept of the clinical feasibility of the Hololens for brain tumor surgery planning in the operating room with quantitative outcome measures. Further development is needed to improve the accuracy and clinical applicability of this wearable mixed reality device.

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# PART III

## Extent of resection and survival





# Chapter 8

## The association between the extent of glioblastoma resection and survival in light of MGMT promoter methylation in 326 patients with newly diagnosed IDH wildtype glioblastoma

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## **Abstract**

### **Background**

Studies on the impact of contrast enhanced (CE) and non-contrast enhanced (NCE) tumor resection in patients with glioblastoma in light of molecular subtypes are limited. The aim of this study was to assess the impact of CE and NCE tumor resection in light of MGMT promoter methylation on survival in newly diagnosed IDH wildtype glioblastoma.

### **Methods**

Patients with newly diagnosed IDH wildtype glioblastoma who underwent surgery were eligible. CE and NCE tumor volumes were assessed on pre- and post-operative MRI scans and extent of resection was calculated. The impact of CE and NCE resection was evaluated using multivariable Cox proportional hazards models and Kaplan Meier analyses.

### **Results**

326 patients were included: 177 (54.3%) with and 149 (45.7%) without MGMT methylation. Multivariable Cox proportional hazards models stratified for MGMT methylation identified age  $\leq 65$ y (HR 0.63; 95% CI, 0.49-0.81;  $p < 0.0001$ ), chemoradiation (HR 0.13; 95% CI, 0.09-0.19;  $p < 0.0001$ ), maximal CE resection (HR 0.58; 95% CI, 0.39-0.87;  $p = 0.009$ ), extended NCE resection (HR 0.71; 95% CI, 0.53-0.93;  $p = 0.014$ ) and minimal residual CE tumor volume (HR 0.64; 95% CI, 0.46-0.88  $p = 0.007$ ) as being associated with longer OS. Kaplan Meier analyses showed that extensive surgery was more beneficial for patients with MGMT methylated glioblastoma.

### **Conclusion**

This study shows an association between maximal CE resection, extended NCE resection, minimal residual CE tumor volume and longer OS in patients with newly diagnosed IDH wildtype glioblastoma. Intra-operative imaging and stimulation mapping may be used to pursue safe and maximal resection.

## Introduction

Patients with glioblastoma have a poor prognosis with a median overall survival of 10 - 15 months, despite safe and maximal surgical resection followed by chemo- and radiotherapy.<sup>(1)</sup> This prognosis varies based on known factors such as age, KPS, extent of resection, isocitrate dehydrogenase (IDH) mutation status, and methylguanine methyltransferase (MGMT) promoter methylation status.<sup>(2,3)</sup>

Maximal resection of the contrast enhanced (CE) portion of glioblastoma has been associated with better overall survival and is currently part of standard surgical glioblastoma treatment.<sup>(3)</sup> However, glioblastoma is known to infiltrate far beyond the margins of CE as seen on MRI, into the surrounding non-contrast enhanced (NCE) edematous T2-weighted or FLAIR abnormality area.<sup>(4)</sup> This raises the question whether maximal CE resection should be extended beyond CE, into NCE area, to improve survival.<sup>(5)</sup> A recent meta-analysis and a systematic review suggested that there is an association between maximal CE resection with resection of NCE and overall survival.<sup>(6,7)</sup> However, the quality of evidence of the available studies was low due to confounding and selection biases. On top, studies investigating the impact of CE and NCE resection have reported limited molecular data on IDH mutation and MGMT promoter methylation of their studied glioblastoma population.<sup>(6,8)</sup>

Thus, in light of the WHO 2016 reclassification, which now includes such molecular data, the impact of CE and NCE glioblastoma resection needs to be re-evaluated in a molecularly homogenous glioblastoma IDH wildtype population, while considering the impact of MGMT promoter methylation.<sup>(9)</sup> The aim of this study therefore, was to assess the impact of CE and NCE tumor resection in light of MGMT promoter methylation on survival in a cohort of patients with newly diagnosed IDH wildtype glioblastoma.

## Methods

### Patients

All consecutive patients aged 18 years or older, newly diagnosed with a CE mass lesion as seen on post-contrast T1-weighted MRI scans, histopathological confirmed as IDH wildtype glioblastoma, who underwent tumor resection or biopsy between January 2012 and May 2018 at [...] were considered for this retrospective study. Patients were eligible if pre- and immediate post-operative (<48 hours) T2-weighted or FLAIR and post-contrast T1-weighted MRI scans were available together with complete molecular data on IDH mutation and MGMT methylation. Molecular analysis was post-hoc performed in patients with unknown IDH mutation or MGMT methylation status; patients without enough tumor material for molecular analysis or in whom assays failed to produce a test result were excluded. The study was approved by the Medical Ethical Committee of Erasmus MC, who waived the need for written informed consent from the patients due to the retrospective

nature of this study and the (emotional) burden that would result from contacting the patients or their relatives to obtain consent. The study was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards and reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

### **Image acquisition, tumor segmentation and extent of resection**

From pre- and post-operative MRI scans, which were obtained in the clinical routine either on a 1.5T or 3.0T scanner, post-contrast T1-weighted and T2-weighted or FLAIR images were collected.

For glioblastoma segmentation, we imported both pre- and post-operative post-contrast T1-weighted and T2-weighted or FLAIR scans into Brainlab (BrainLab, Feldkirchen, Germany, version 2.1.0.15). Using the SmartBrush tool in Brainlab Elements, we semi-automatically segmented all tumor involved CE on pre-operative post-contrast T1-weighted scans (including the necrotic part, if present) and all tumor involved CE on post-operative post-contrast T1-weighted scans (excluding small vessels in the surgical cavity or hemorrhage). We then semi-automatically segmented all tumor-related NCE on both pre- and post-operative T2-weighted or FLAIR scans (excluding extra-lesional hemorrhage). We attempted to minimize the inclusion of surgery induced new T2-weighted or FLAIR abnormality by overlaying and carefully comparing pre- and post-operative MRIs. We manually corrected all segmentations when needed using the manual Brush tool. All tumor volumes were assessed while being blinded for patients' clinical outcome.

We finally obtained four tumor volumes (cm<sup>3</sup>): pre-operative and residual CE volumes and pre-operative and residual NCE volumes. We calculated the CE surrounding NCE volumes by subtracting CE volumes from the total NCE volumes. We calculated the extent of resection (EOR, %) separately for both the CE and NCE portion with the formula: [(pre-operative volume – residual volume)/pre-operative volume] \* 100.<sup>(10)</sup> Maximal CE resection was categorized in our dataset as CE EOR >97% and extended NCE was categorized as NCE resection of ≥ 30% based on threshold analysis (Figure 1 and 2 of Supplementary Material). Tumors that were biopsied were segmented only on pre-operative MRI scans and their EOR was imputed as being 0%.

### **Molecular analysis**

Tumor tissue samples were obtained from patients through surgical resection or biopsy. Histopathological examination was performed by neuropathologists; IDH mutational analysis was assessed with sequencing and MGMT methylation status with a methylation specific PCR, as described elsewhere.<sup>(11-13)</sup>

## Statistical analysis

Overall survival (OS) was defined as time from surgery to death (primary outcome) and progression-free survival (PFS) was defined as time from surgery till clinical or radiological progression (secondary outcome). Patients were censored at time of last clinical follow up date.

Statistical analyses were performed with SPSS 25.0 statistical software (IBM Corp.). Pre-operative and post-operative residual tumor volume distributions were skewed and therefore log transformed prior to statistical analysis. Descriptive statistics were tested between MGMT methylation status groups with the Chi Squared Test or Fisher Exact test in case of categorical variables, with the Kruskal Wallis test in case of continuous non-normal distributed data and with log rank tests to compare median OS and PFS when using Kaplan Meier analysis.

The associations between each variable and outcome were first tested with univariable Cox proportional hazards models and all variables with  $p < 0.10$  (entry significance threshold) were selected for multivariable Cox proportional hazards models. These models were stratified for MGMT, because this variable violated the proportional hazards assumption. Hazard Ratio's (HR) with 95% Confidence Intervals (CI) were estimated for each variable within the model. A  $p$ -value  $< 0.05$  was considered statistically significant.

## Results

There were 375 glioblastoma patients considered for this study. We excluded 36 (9.6%) due to insufficient tissue material for molecular analysis, and 13 (3.5%) because of the presence of IDH mutation. In total, 326 IDH wildtype glioblastoma were included in our analysis: 177 (54.3%) with and 149 (45.7%) without MGMT promoter methylation. Maximal CE resection was achieved in 61 patients (18.7%), while in 187 (57.4%) patients maximal resection of CE could not be achieved. Seventy-eight patients (23.9%) underwent biopsy. Extended NCE was achieved in 156 patients (47.9%) and no or limited NCE resection was performed in 170 patients (52.1%). Median OS and PFS was 309 days (95% CI, 278-340) and 174 days (95% CI, 159-209) respectively. Further patient and tumor characteristics are presented in Table 1.

**Table 1.** Patient and tumor characteristics.

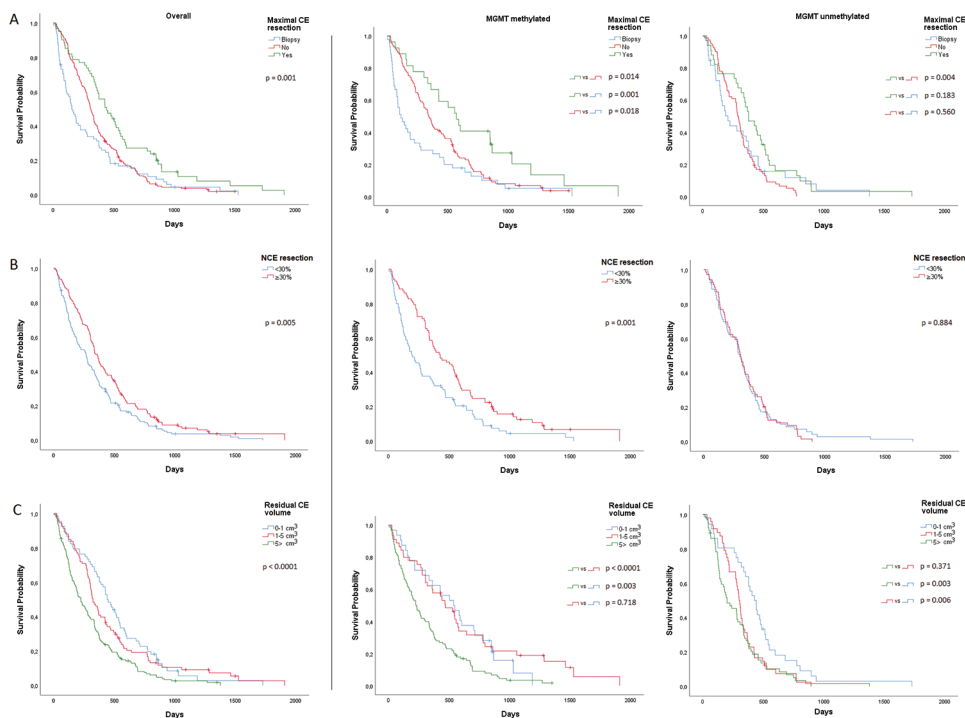
	MGMT promoter						p value
	All		Methylated		Unmethylated		
	n	%	n	%	n	%	
Characteristics	326	100	177	54.3	149	45.7	
Sex							0.006
Male	206	63.2	100	48.5	106	51.5	
Female	120	36.8	77	64.2	43	35.8	
Age, years							0.122
≤ 65	162	49.7	81	50.0	81	50.0	
> 65	164	50.3	96	58.5	68	41.5	
Mean, years (SD)	63.8 (10.5)		64.2 (10.9)		63.3 (10.1)		0.453
KPS							0.748
≤ 70	119	36.5	66	55.5	53	45.5	
> 70	207	73.5	111	53.6	96	46.4	
Mean (SD)	79.2 (12.4)		78.8 (12.2)		79.7 (12.6)		0.480
Pre-operative tumor volume, median cm <sup>3</sup> (IQR)							
CE	34.9 (15.5-55.8)		30.7 (13.8-52.5)		39.9 (17.1-26.2)		0.050
NCE	72.1 (29.4-127.5)		78.1 (27.8-133.8)		64.9 (32.3-113.4)		0.249
Residual tumor volume, median cm <sup>3</sup> (IQR)							
CE	5.0 (1.41-12.3)		6.1 (1.8-12.5)		3.8 (1.1-12.0)		0.097
NCE	39.7 (18.3-73.7)		42.2 (17.1-79.6)		36.5 (20.4-64.2)		0.699
Maximal CE resection							0.213
Yes	61	18.7	27	44.3	34	55.7	
No	187	57.4	105	56.2	82	43.8	
Biopsy	78	23.9	45	57.7	33	42.3	
Median EOR (IQR)	83.4 (14.2-94.8)		79.6 (0-91.9)		88.0 (25-7-96.0)		0.075
NCE resection							0.609
≥ 30%	156	47.9	87	55.8	69	44.2	
< 30%	170	52.1	90	52.9	80	47.1	
Median EOR (IQR)	27.1 (0-57.1)		28.7 (0-58.4)		25.8 (0-55.0)		0.948
Adjuvant therapy							0.294
No therapy	61	18.7	38	62.3	23	37.7	
Radio- or chemotherapy alone	56	17.2	27	48.2	29	51.8	
Chemoradiation	209	64.1	112	58.4	97	41.6	
Overall patient outcome	x	x	x	x	x	x	x
Median overall survival (95% CI)	309 (278.0-340.0)		334 (266.8-401.2)		305 (275.2-334.8)		0.003
Median progression-free survival (95% CI)	184 (159.3-208.7)		174 (131.2-216.8)		190 (162.0-218)		0.053

MGMT methylguanine methyltransferase, SD standard deviation, CE contrast enhancement, NCE non contrast enhancement, EOR extent of resection, IQR Inter Quartile Range, CI Confidence interval.

Univariable Cox proportional hazards regression analysis identified age  $\leq 65$ y (HR 0.64; 95% CI, 0.51-0.80;  $p < 0.0001$ ), KPS  $>70$  (HR 0.59; 95% CI, 0.47-0.74;  $p < 0.0001$ ), MGMT promoter methylation (HR 0.72; 95% CI, 0.57-0.90;  $p < 0.004$ ), adjuvant chemoradiation (HR 0.14; 95% CI, 0.11-0.19;  $p < 0.0001$ ), smaller pre-operative CE tumor volumes (per  $\text{cm}^3$  HR 0.92; 95% CI, 0.83-1.01;  $p < .094$ ), maximal CE resection (HR 0.51; 95% CI, 0.36-0.72;  $p < 0.0001$ ), and extended NCE resection (HR 0.72; 95% CI, 0.58-0.91;  $p = 0.005$ ) as being associated with longer OS. As further presented in Table 2, the variables age  $\leq 65$ y, KPS  $>70$ , adjuvant chemoradiation, maximal CE resection and extended NCE resection were also significantly associated with a longer PFS in univariable Cox regression analysis ( $p < 0.05$ ). Kaplan Meier curves for OS and PFS for each variable are presented in Figure 3 of Supplementary Material.

Multivariable Cox proportional hazards regression analysis stratified for MGMT methylation status and risk adjusted for age  $\leq 65$ y (HR 0.63; 95% CI, 0.49-0.81;  $p < 0.0001$ ), KPS  $>70$  (HR 0.92; 95% CI, 0.71-1.19;  $p = 0.545$ ), adjuvant chemoradiation (HR 0.13; 95% CI, 0.09-0.19;  $p < 0.0001$ ), and smaller pre-operative CE tumor volumes per  $\text{cm}^3$  (HR 0.85; 95% CI, 0.75-0.95;  $p = 0.007$ ) identified maximal CE resection (HR 0.58; 95% CI, 0.39-0.87;  $p = 0.009$ ) and extended NCE resection (HR 0.71; 95% CI, 0.53-0.93;  $p = 0.014$ ) as being associated with longer OS (Table 2). Variables that remained significantly associated with a longer PFS were KPS  $>70$  (HR 0.59; 95% CI, 0.45-0.77;  $p < 0.0001$ ) and adjuvant chemoradiation (HR 0.09; 95% CI, 0.06-0.14;  $p < 0.0001$ ). Explorative multivariable Cox proportional hazards regression analyses showed that higher NCE resection thresholds (e.g.  $\geq 50\%$ ) were not associated with a favorable OS ( $p > 0.05$ ) (for threshold analysis, see Figure 1 of Supplementary Material).

The impact of maximal CE resection on survival was more beneficial for patients with MGMT methylated glioblastoma and significantly improved median OS (572 days; 95% CI, 424-720), when compared to STR (342 days; 95% CI, 282-402;  $p = 0.014$ ) or biopsy (112 days; 95% CI, 36-42;  $p = 0.001$ ) (Figure 1A). Patients with MGMT methylated glioblastoma also had a longer OS with extended NCE resection (425 days; 95% CI, 286-564) than when extended NCE was not achieved (190 days; 95% CI, 107-273;  $p = 0.001$ ) (Figure 1B). In patients with MGMT unmethylated glioblastoma, no survival benefit was observed with extended NCE resection ( $p = 0.884$ ).



**Figure 1.** Overall and MGMT-stratified Kaplan Meier curves for overall survival A) Maximal CE resection B) Extended NCE resection and C) Minimal residual volume.

We further assessed minimal post-operative CE residual volumes with a potential positive impact on OS. In MGMT methylated glioblastoma, when compared to  $>5 \text{ cm}^3$  residual CE tumor (224 days; 95% CI 164-284), we observed a significantly longer median OS for a residual CE tumor volume of  $1-5 \text{ cm}^3$  (470 days; 95% CI 330-610;  $p < 0.0001$ ) and  $0-1 \text{ cm}^3$  (536 days; 95% CI 319-752;  $p = 0.003$ ) (Figure 1C). In MGMT unmethylated glioblastoma, we only observed a longer OS in residual CE volumes of  $0-1 \text{ cm}^3$  (427 days; 95% CI, 350-503), when compared to  $1-5 \text{ cm}^3$  (299 days; 95% CI, 270-327;  $p = 0.006$ ) or to  $>5 \text{ cm}^3$  (200 days; 95% CI, 102-298;  $p = 0.003$ ) (Figure 1C). In these tumors, no difference in median OS was observed between  $1-5 \text{ cm}^3$  and  $>5 \text{ cm}^3$  ( $p = 0.371$ ) residual CE tumor volume. Multivariable Cox proportional hazards regression analysis stratified for MGMT and adjusted for age, KPS, adjuvant chemoradiation and pre-operative CE tumor volume, identified a residual CE tumor volume of  $0-1 \text{ cm}^3$  (HR 0.64; 95% CI, 0.46-0.88  $p = 0.007$ ) and  $1-5 \text{ cm}^3$  (HR 0.71; 95% CI, 0.54-0.94;  $p = 0.016$ ) as being associated with favorable OS. These analyses were also performed for post-operative residual NCE volumes, but here no minimal volume threshold with a positive impact on OS was identified.

Table 2. Cox proportional hazards models for overall survival and progression-free survival.

Variable	Overall survival				Progression-free survival			
	Median days, 95%CI	Univariable HR, 95% CI	P Value #	Adjusted HR, 95% CI	P Value #	Univariable HR, 95% CI	Adjusted HR, 95% CI	P Value \$
<b>Age, years</b>								
> 65	259, 191-327	a						
≤ 65	358, 311-405	0.64, 0.51-0.80	< 0.0001	0.63, 0.49-0.81	< 0.0001	0.70, 0.56-0.89	0.82, 0.64-1.06	0.123
<b>KPS</b>								
≤ 70	199, 142-256	a				a		
> 70	369, 319-419	0.59, 0.47-0.74	< 0.0001	0.92, 0.71-1.19	0.545	0.51, 0.40-0.6	0.59, 0.45-0.77	< 0.0001
<b>MGMT promoter</b>								
Unmethyalted	305, 275-335	a		b		a	b	
Methyalted	334, 267-401	0.72, 0.57-0.90	0.004			0.79, 0.62-1.00		0.054
<b>Adjuvant therapy</b>								
None	65, 46-84	a				a		
Radiotherapy or chemotherapy	217, 146-288	0.38, 0.26-0.55	< 0.0001	0.29, 0.19-0.43	< 0.0001	0.23, 0.15-0.36	0.22, 0.14-0.35	< 0.0001
Chemoradiation	423, 367-479	0.14, 0.11-0.19	< 0.0001	0.13, 0.09-0.19	< 0.0001	0.09, 0.06-0.13	0.09, 0.06-0.14	< 0.0001
<b>Pre-operative CE tumor volume</b>								
smaller per cm <sup>3</sup>		0.92, 0.83-1.01	0.094	0.85, 0.75-0.95	0.007	0.94, 0.85-1.04	0.99, 0.88-1.11	0.860
<b>Maximal CE resection</b>								
*						*		
Biopsy	153, 106-200	a				a		
No	309, 278-340	0.79, 0.60-1.04	0.090	0.87, 0.61-1.23	0.415	0.80, 0.61-1.05	1.25, 0.89-1.81	0.228
Yes	441, 321-560	0.51, 0.36-0.72	< 0.0001	0.58, 0.39-0.87	0.009	0.59, 0.42-0.85	0.97, 0.63-1.50	0.898
<b>NCE resection</b>								
*						*		
< 30%	266, 213-319	a				a		
≥ 30%	344, 304-385	0.72, 0.58-0.91	0.005	0.71, 0.53-0.93	0.014	0.82, 0.65-1.03	0.87, 0.65-1.15	0.325

<sup>a</sup> Reference group <sup>b</sup> Proportional hazard assumption for MGMT was violated, therefore multivariable Cox regression model was stratified for MGMT. HR Hazard ratio, MGMT methylguanine methyltransferase, CI Confidence Interval \*for MGMT-stratified median overall survival, see Figure 1 # entry significance threshold of p < 0.1 \$ significance threshold of p < 0.05



## Discussion

This study shows an association between maximal CE resection, extended NCE resection, minimal residual CE tumor volume and longer OS in 326 patients with newly diagnosed IDH wildtype glioblastoma. We observed that extensive resection was more beneficial for patients with MGMT methylated IDH wildtype glioblastoma.

Maximal resection of CE has earlier been associated with longer OS in a large meta-analysis of neurosurgical literature based on 37 studies and 41,117 unique glioblastoma patients.<sup>3</sup> This association has recently been re-evaluated based on two new insights. First, studies performed before the WHO 2016 reclassification have included limited molecular data, because the impact of molecular subtyping of glioblastoma according to IDH mutation status was less of a consideration.<sup>(3,8,14-16)</sup> Although IDH mutation within newly diagnosed primary glioblastoma is rare (<5%, and 3.5% in our cohort) these tumors represent a distinct molecular type of glioma arising from a distinct precursor lesion.<sup>(17,18)</sup> Therefore, incomplete or absence of molecular data on IDH mutation and MGMT methylation or mixing molecular subtypes when evaluating the impact of glioblastoma resection on survival is undesirable. More recent studies did investigate the impact of glioblastoma surgery on survival in light of molecular markers. Senft et al. studied the impact of maximal CE resection and MGMT promoter methylation status in a homogenous IDH wildtype glioblastoma population (n = 175) and showed that both were significantly associated with longer OS.<sup>(2)</sup> Ellingson et al. showed in 1,054 glioblastoma patients (with partially available data on IDH mutation and MGMT methylation) that smaller residual CE tumor volumes (<12 cm<sup>3</sup>) and MGMT methylation were significantly associated with longer OS in patients receiving chemoradiation.<sup>(19)</sup> A recent study published by Molinaro et al confirmed the association between maximal CE resection and OS across all molecular subgroups of glioblastoma.<sup>(20)</sup>

Secondly, the association between glioblastoma resection and OS is also being reassessed by evaluating the value of NCE resection, because it is known that glioblastoma infiltrates beyond the margins of CE into the NCE area.<sup>(4)</sup> This aspect of glioblastoma surgery is also recently investigated by Molinaro et al.<sup>(20)</sup> The authors found that maximal resection of CE and NCE tumor was associated with longer OS in younger patients with IDH wildtype glioblastoma regardless of MGMT methylation status (subset of 190 patients with known IDH mutation and MGMT methylation status). In this study, maximal NCE resection that was associated with OS was defined as 92% NCE resection after maximal CE resection. Such an extensive surgical approach may only safely be achievable with use of intra-operative imaging, fluorescent guidance or stimulation mapping.<sup>(21,22)</sup> Other studies have associated lower NCE resection thresholds of 53% and 45% with OS.<sup>(8,23)</sup> We observed that a NCE resection threshold of 30% was associated with OS. In exploratory threshold analysis, higher thresholds (e.g. minimal ≥60% NCE tumor resection) seemed not to be associated with OS anymore. This may suggest that resection of NCE tumor immediately

surrounding CE improves survival, but extending the resection on further distance from CE into NCE tumor does not provide survival benefit. It can be hypothesized that the direct peritumoral NCE area reflects a higher degree of tumor infiltration than the NCE area further away from the CE tumor, which is presumably more dominated by edema than tumor infiltration.<sup>(4)</sup> In future research, a combination of physiological imaging modalities - such as MR spectroscopy, diffusion and perfusion imaging or positron-emission tomography - may be used to more accurately detect tumor infiltrated portions in NCE and to tailor surgical planning.<sup>(24)</sup>

### Limitations

The main limitation of this study is its retrospective nature. This may have introduced some degree of selection bias. We attempted to limit selection bias by consecutive inclusion of all glioblastoma patients operated upon between 2012 and 2018 in our cohort, including patients with complex glioblastoma localization (crossing midline or deep within the basal ganglia) who underwent diagnostic biopsies. We also performed IDH mutational and MGMT promoter methylation analyses on all glioblastoma included in our cohort. A second limitation is that only one observer assessed both pre- and post-operative tumor volumes. In this context, a stringent assessment of residual volumes in the resection cavity by one observer may explain the relatively low maximal CE resection percentage of 17.8%. Although the interobserver agreement is high for pre-operative volumes, it is known to be relatively low for residual tumor volumes. The intra-observer agreement nevertheless, is known to be high for both pre-operative as residual tumor volumes.<sup>(10,25)</sup> We have also attempted to limit bias during volumetric assessment by blinding the assessor for patients' clinical outcome. In future research, our findings need to be validated in an external validation set.

### Conclusion

This study shows an association between maximal CE resection, extended NCE resection, minimal residual CE tumor volume and longer OS in patients with newly diagnosed IDH wildtype glioblastoma. Extensive resection was more beneficial for patients with MGMT methylated glioblastoma. Intra operative imaging and stimulation mapping may be used to pursue maximal CE resection and extended NCE resection.

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Supplementary materials

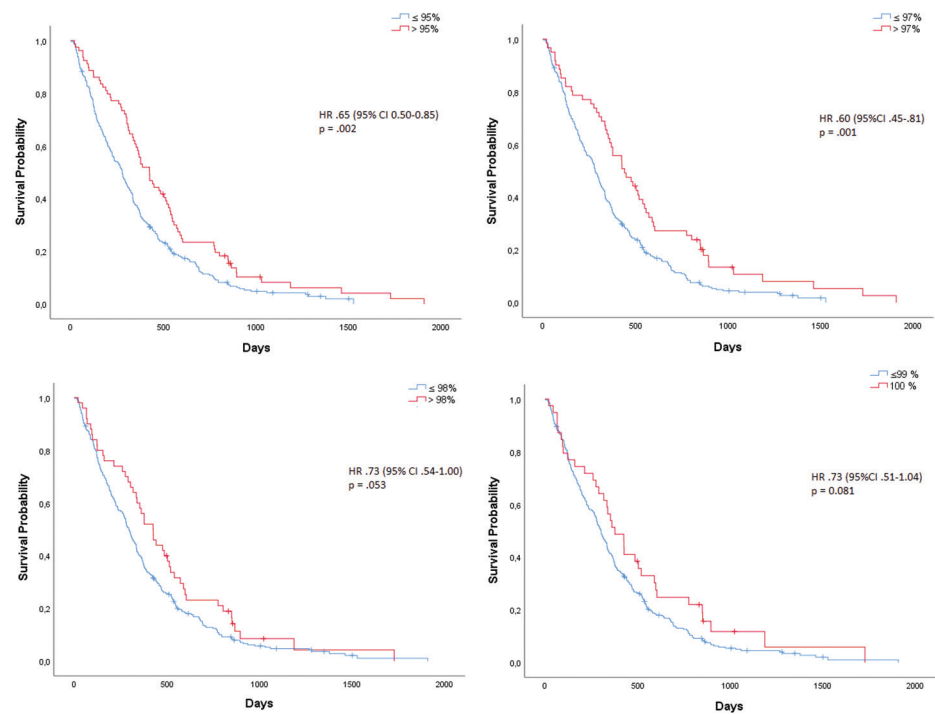


Figure S1. Kaplan Meier curves of OS for different EOR thresholds.

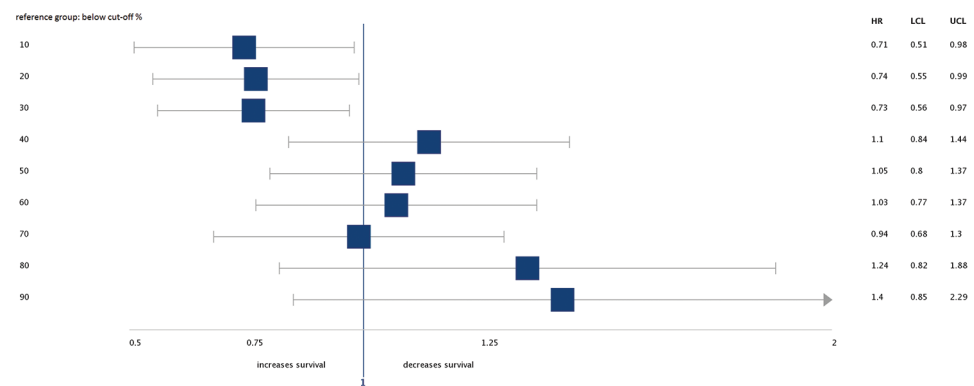


Figure S2. Hazard ratios with 95% CI for different NCE resection thresholds in explorative multivariable Cox proportional hazards models (stratified for MGMT methylation status, adjusted for age, KPS, adjuvant therapy, EOR and pre-operative tumor volume).

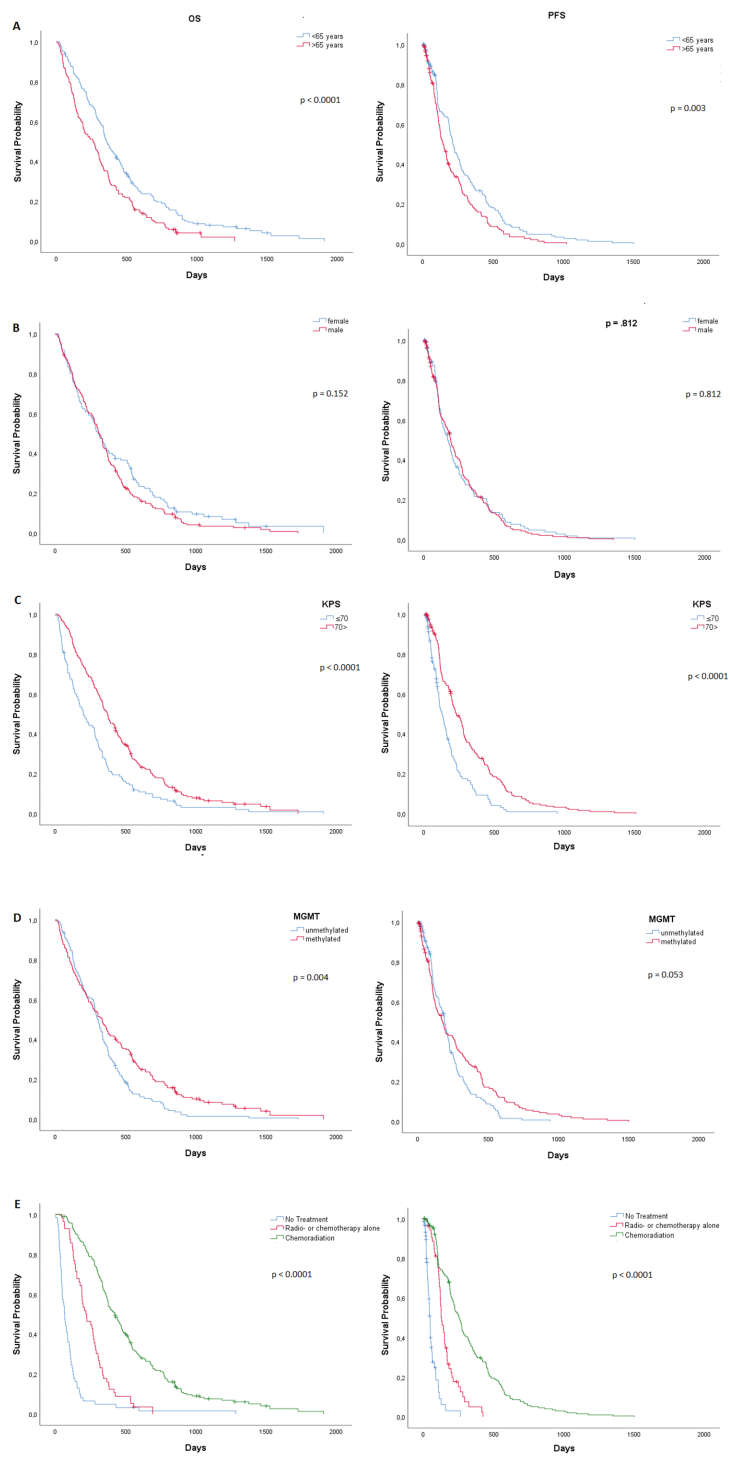


Figure S3. Kaplan Meier curves of OS and PFS for (A) age, (B) gender, (C) KPS, (D) MGMT promotor methylation, (E) adjuvant therapy.





# Chapter 9

## Development and external validation of a clinical prediction model for survival in glioblastoma patients

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*Submitted*





# Chapter 10.1

## Association between supratotal glioblastoma resection and patient survival: a systematic review and meta-analysis

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*World Neurosurg.* 2019 Jul;127:617-624

## **Abstract**

### **Background**

Gross total resection (GTR) of contrast enhancement (CE) improves survival of glioblastoma (GBM) patients. However, GBM infiltrates into brain parenchyma, beyond CE. It remains unclear whether resection beyond CE (supratotal resection, SPTR) improves survival without causing additional neurological deficits. The aim of this meta-analysis was to study the association between SPTR and overall survival in GBM patients.

### **Methods**

Embase, Pubmed and other literature databases were searched for eligible studies until August 2018. Studies involving patients with GBM comparing SPTR with GTR were included in this study. The main outcome was overall survival as hazard ratios (HR) with 95% confidence interval (CI) and median overall survival differences with 95% CI.

### **Results**

Meta-analysis based on six studies and 1,168 unique patients with GBM showed that when compared with GTR, SPTR of GBM resulted in 53% lower risk of mortality at any time during follow-up (HR = 0.47, 95% CI = 0.31 - 0.72, P = 0.0005). The median overall survival of SPTR was 6.4 months (95% CI = 3.2 - 9.7) longer than GTR (P = 0.0001). Reports on post-operative deficits were limited and the quality of evidence was moderate to very low.

### **Conclusion**

When compared with GTR, SPTR of GBM suggests lower risk of mortality and longer median overall survival. However, the quality of evidence of the available studies was poor. Therefore, it remains unclear whether SPTR is safe and whether it really improves survival of patients with GBM. Future prospective trials and a standardized definition of SPTR are needed.

## Introduction

Patients with glioblastoma (GBM) have a poor prognosis with a median overall survival of 15 months, despite safe and maximal surgical resection followed by chemo- and/or radiotherapy.<sup>(1)</sup> GBM typically appears on a post-contrast MRI scan as a contrast enhancing (CE) tumor with central necrosis. Maximal and safe surgical resection or gross total resection (GTR) of the CE area is currently the main goal of GBM surgery. Both the survival benefit of GTR and the optimal way to achieve this are extensively debated in the neurosurgical literature.

Brown et al. showed in a systematic review and meta-analysis of 37 articles and 41,117 unique patients, that in comparison with subtotal resection, GTR of CE area decreases the risk of mortality with 28% at 1 year and 16% at 2 years ( $P < 0.001$ ).<sup>(1)</sup> Jenkinson et al. recently showed in a Cochrane review that resection with 5-Aminolevulinic Acid (5-ALA) fluorescence guidance or intra-operative MRI guidance may increase the extent of GBM resection.<sup>(2)</sup>

However, GBM is known to infiltrate far beyond the margins of CE as seen on MRI, into the surrounding edematous T2-FLAIR hyperintense region.<sup>(3)</sup> This raises the question whether GTR of the CE portion is indeed a 'total' resection, or whether surgical resection should also include –part of the- hyperintense T2-FLAIR region to improve survival. It is suggested that 5-ALA fluorescence accumulates in cancer cells and not only corresponds with the CE portion on MRI but also exceeds this area as vague fluorescence, which corresponds with portions of GBM often infiltrating into eloquent brain area.<sup>(4)</sup>

This concept of so-called supramarginal or supratotal resection (SPTR), is already known in the field of low grade glioma surgery,<sup>(5,6)</sup> because low grade gliomas commonly don't enhance and therefore lack the CE target for surgical resection. However, SPTR of GBM is less extensively investigated, and up until this date no quantitative data analysis has been performed to clarify the association of survival with SPTR versus GTR in GBM patients, nor of associated post-surgical neurological complications.

The aim of this study was to investigate the association between SPTR and survival in glioblastoma patients, using a systematic review and meta-analysis in accordance with the PRISMA guidelines.<sup>(7)</sup>

## Methods

### Systematic review

We searched for studies that included GBM patients who had received surgical resection, with pre- and post-operative MRI imaging. Letters, editorials, abstracts and non-English citations were excluded. The search query was designed together with an expert librarian at the Erasmus University Medical Center, Medical Library to capture all citations published until August 2018 within Pubmed [(Glioblastoma/ OR glioblastom\* OR (maligna\* ADJ3

*glioma\*) OR (high\* ADJ3 grade\* ADJ3 glioma\*) OR ((grade-iv OR grade-4) ADJ3 glioma\*) OR gbm).ab,ti,kw.) AND (Surgical Procedures, Operative/ OR exp Neurosurgery/ OR Neurosurgical Procedures/ OR exp Brain Neoplasms/su OR (surg\* OR neurosurg\* OR resect\*).ab,ti,kw.) AND (Margins of Excision/ OR (flair OR (Fluid ADJ3 attenuat\* ADJ3 invers\* ADJ3 recover\*) OR t2 OR t-2 OR gross-total OR ((exten\* OR Supratotal\* OR Supramaxim\*) ADJ3 (resect\* OR remov\*)) OR ((surg\* OR excis\* OR resect\*) ADJ3 margin\*) OR ((beyond OR additional\*) ADJ6 (contrast OR boundar\*))).ab,ti,kw.) NOT (letter\* OR news OR comment\* OR editorial\* OR congres\* OR abstract\* OR book\* OR chapter\* OR dissertation abstract\*).pt.] Embase, Web of Science, Cochrane Central and Google Scholar (search queries for these sources can be found in the Supplementary Materials).*

After removing duplicate articles, two independent reviewers (F.I. and S.K.) screened the articles based on title and abstract, removing off-topic citations. Full texts of remaining articles were independently read by the reviewers to determine whether they were eligible for final inclusion. Articles that studied solely pediatric or non-glioblastoma cases, recurrent glioblastoma, non-surgery or surgery of enhancing tumor portion alone, biopsy-only or articles without survival data were excluded. The study was performed and presented according to the PRISMA guidelines.<sup>(7)</sup>

### Data collection

Main outcome of interest was overall survival as hazard ratio (HR) with 95% confidence interval (CI) and median overall survival with 95% CI of both GTR and SPTR groups. These data were collected from the included studies or calculated based on other available data or extracted based on data points created by a pixel-by-pixel method from survival curves. GTR and SPTR were defined by the authors of the studies that were included in our meta-analysis. We categorized GTR as 100% resection of CE and SPTR as every effort of resection beyond GTR of CE, which was qualitatively or quantitatively defined by authors of the included studies. Li et al. presented their data on survival and neurological outcome mainly by categorizing <53 % FLAIR resection and ≥53% FLAIR resection. However, after email correspondence with the senior author, we received additional survival data on the GTR and SPTR groups to perform our meta-analysis.<sup>(8)</sup>

When available, data on post-operative new neurological deficits, surgical complications, or quality of life after surgery was collected to assess the safety of both SPTR and GTR.

### Meta-analysis

HR with 95% CI and median overall survival with 95% CI of both SPTR and GTR groups were collected or calculated for each study based on available data using the random effects model and presented in forest plots using Review Manager (RevMan, version 5.3; Cochrane Collaboration). The random effects model was used instead of the fixed effects model due to heterogeneity between the studies, in order to give a more conservative and a clinically

reliable interpretation of the summarized statistics and the confidence intervals. The HRs were corrected by the authors of the individual papers for several prognostic factors across studies such as age, Karnofsky Performance Status (KPS), adjuvant therapy, tumor volume and location and methylguanine-DNA methyltransferase (MGMT) methylation and isocitrate dehydrogenase (IDH) mutation status (see Supplementary Materials for details). A p value of  $< 0.05$  was considered to be statistically significant. Heterogeneity was calculated and interpreted using the Chi-squared test and  $I^2$  values with Review Manager.

### Quality of evidence

Quality of evidence obtained from the articles in this study was graded based on the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) system.<sup>(9)</sup> Within this system, four levels of quality rating can be assigned to studies; from 'high', often given to randomized controlled trials, to 'moderate', 'low' often given to observational studies, and 'very low'. The quality of evidence was rated by F.I. and was based on methodological quality, risk of biases (based on ROBINS-I),<sup>10</sup> heterogeneity, and precision of effect estimates.

### Results

We identified 1,796 citations from Embase, 1,503 from Pubmed, 1,424 from Web of Science, 140 from Cochrane Central and 200 from Google Scholar. Removal of duplicate articles resulted in 2,346 unique citations that were screened based on title and abstracts. In total, 2,322 off-topic articles were excluded, leaving 24 articles that were read in full.<sup>(6,8,11-32)</sup> After reading, 18 articles were excluded, due to absence of SPTR (N=11), absence of sufficient survival data (N=4), and article type (N=3; two commentaries and one review). This resulted in a final total of six studies<sup>(8,12,18,19,21,28)</sup> that were included in our systematic review and meta-analysis. The process of study inclusion is presented in the PRISMA flow diagram in Figure 1 and demographics of the included studies are presented in Table 1.

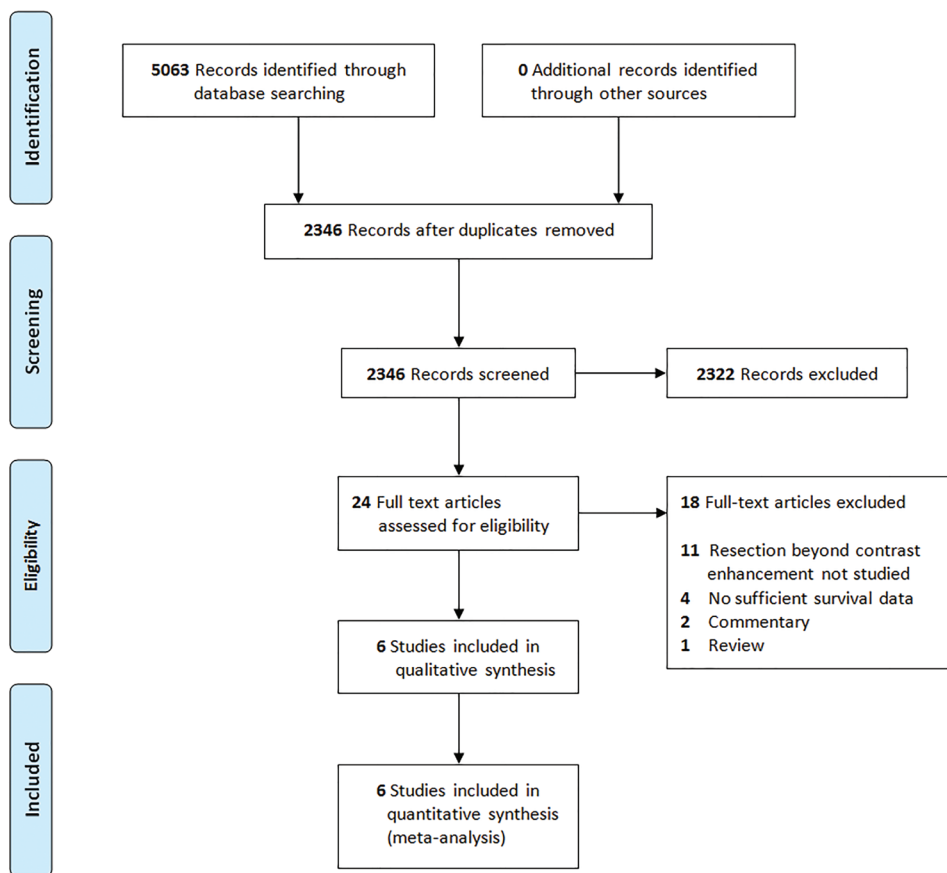


Figure 1. PRISMA Flow Diagram.

Table 1. Study demographics.

Study	Country	GTR defined as resection of	SPTR defined as resection of	HR: group comparison*	HR: multivariate analysis†	Quality of evidence (GRADE)‡
Aldave 2013 <sup>(12)</sup>	Spain	100% of CE	100% of CE + total resection of fluorescing tumor	Yes	Yes	3
Li 2016 <sup>(8)</sup>	United States	100% of CE	100% of CE + >0% to 100% FLAIR	Yes	Yes	2
Eyupoglu 2016 <sup>(19)</sup>	Germany	100% of CE	100% of CE + total resection of fluorescing tumor	Yes	No	3
Pessina 2017 <sup>(28)</sup>	Italy	100% of CE	100% of CE + 100% of FLAIR	Yes	Yes	3
Esquanazi 2017 <sup>(18)</sup>	United States	95-100% of CE	>100% of CE	Yes	Yes	3
Glenn 2018 <sup>(21)</sup>	United States	100% of CE	100% of CE + > 1 cm surrounding brain tissue	Yes	Yes	4



GTR: Gross total resection, SPTR: Supra total resection, CE: Contrast enhancement, HR: Hazard Ratio \* Yes: HR should be interpreted as a between group comparison, No: HR should be interpreted as per unit of residual T2-FLAIR volume

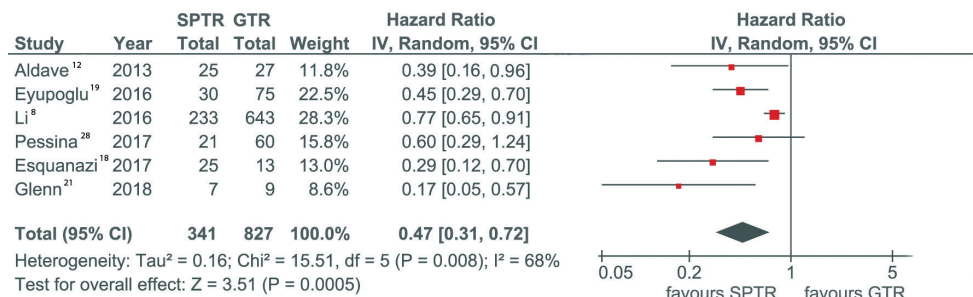
† Which included prognostic factors in the model such as age, tumor volume, tumor location, Karnofsky Performance Status, MGMT methylation status and adjuvant therapy. For a full overview, see Supplementary Materials.

‡ Levels of quality: 1: high, 2: moderate, 3: low, 4: very low. For details, see Supplementary Materials.

## Meta-analysis

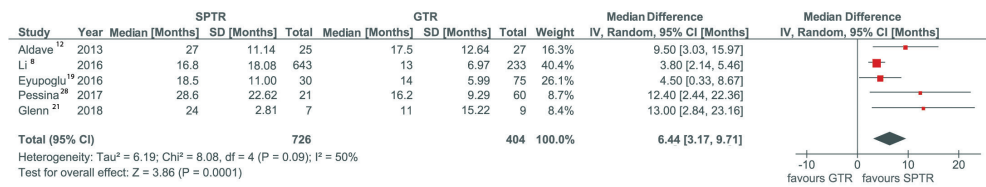
Our meta-analysis based on six studies comparing SPTR with GTR<sup>(8,12,18,19,21,28)</sup> included 1,168 unique patients with GBM and showed that when compared with GTR, SPTR of GBM resulted in 53% lower risk of mortality at any time during follow up (HR = 0.47, 95% CI = 0.31 - 0.72, P = 0.0005, I<sup>2</sup> = 68%) (Figure 2).

The median overall survival for GTR and SPTR was 15.0 months (95% CI = 2.9 – 26.1) and 28.3 months (95% CI = 12.5- 44.1), respectively (P < 0.0001). The survival benefit with SPTR was 13.3 months (95% CI = 3.6 - 23.1) when compared with GTR (P = 0.007). However, Esquanazi et al.<sup>(18)</sup> especially influenced the heterogeneity (I<sup>2</sup>) of the median overall survival analysis, possibly due to a much higher median overall survival for SPTR of 54 months within this study. Exclusion of this study in an additional median overall survival analysis showed a decrease of I<sup>2</sup> from 96% to 50%. When compared with GTR, SPTR of GBM resulted in a longer median overall survival of 6.4 months (95% CI = 3.2 - 9.7, P = 0.0001) (Figure 3).



**Figure 2.** Forest plot with hazard ratio and 95% confidence interval (CI) for supratotal resection (SPTR) versus gross total resection (GTR).





**Figure 3.** Forest plot with median overall survival difference between supratotal resection (SPTR) versus gross total resection (GTR).

### Quality of evidence

No randomized controlled trials were available for inclusion in this meta-analysis; all studies were retrospective with a non-randomized treatment assignment. Following the GRADE guidelines, studies were assigned as ‘low’ level<sup>(12,18,19,28)</sup> quality evidence, upgraded to a ‘moderate’ level<sup>(8)</sup> or downgraded to a ‘very low’ level<sup>(21)</sup> (Table 1) based on previously mentioned mainly methodological factors such as study design, internal validity, risk of biases, and precision (for details; see Supplementary Materials).

### Post-operative new neurological deficits

While in three studies incidence rates of post-operative new neurological deficits for both SPTR (0%-19%) and GTR (0%-8%) groups were reported, in other three studies this data was either not (clearly) presented or absent (Table 2). Overall, reports were limited and based on this data the difference in post-operative neurological outcome between SPTR vs GTR could not clearly be analyzed.

**Table 2.** Reported incidence rates of post-operative neurological deficits.

Study	GTR N (%)	SPTR N (%)	P value	Comments
Aldave 2013 <sup>(12)</sup>	2 (8)	4 (18.5)	0.27	No details on the deficits was reported.
Li 2016 <sup>(8)</sup>	NA	120 (19)	NA	Motor deficits, speech and visual impairments were the most commonly reported 30-day post-operative neurological complications.
Glenn 2018 <sup>(21)</sup>	0 (0)	1 (11.1)	0,66	One patient with dysphasia.
Pessina 2017 <sup>(28)</sup>	0 (0)	0 (0)	NA	There were no patients that developed post-operative new neurological deficits in the GTR and SPTR groups.
Eyupoglu 2016 <sup>(19)</sup>	see comments		0.47 – 1.0	Rates on post-operative new neurological deficits between groups was not clearly collectable. Overall no significant worsening of motor, visual, speech, cognitive deficits or seizures was reported in both groups.
Esquanazi 2017 <sup>(18)</sup>	NA	NA	NA	3 (7.9%) had transient and 2 (5.3%) had permanent neurological deficits of all 38 patients. Group-specific data was not reported.

NA: not applicable.

## Discussion

This PRISMA guided systematic review and meta-analysis studied the association between SPTR and survival in patients with GBM. This study based on six articles and 1,168 unique patients showed that when compared with GTR, SPTR of GBM resulted in 53% lower risk of mortality at any time during follow-up, and 6.4 months longer median overall survival. The quality of evidence of the available studies, however, was moderate to very low. Furthermore, the incidence rates of post-operative new neurological deficits were minimally and only qualitatively reported, and data on quality of life were not reported in any of the included studies. It therefore remains unclear whether SPTR can be achieved as safely as GTR.

### Defining SPTR

There are a multitude of definitions for SPTR. The term was first coined by Yordanova et al. in 2011 to explain the procedure of low grade glioma resection guided by functional boundaries, defined by the area of T2-FLAIR hyperintensity<sup>(33)</sup> during awake surgery, instead of classical neuronavigation based pre-operative MRI post-contrast scans. The authors showed that SPTR of low-grade glioma resulted in significant delay of anaplastic degeneration ( $P = 0.037$ ) without causing permanent neurological deficits.

With SPTR of GBM, the classically used CE boundaries of the tumor are exceeded to include the non-enhancing hyperintense T2-FLAIR region in the resection, presumed to represent edematous, tumor infiltrated tissue. This region can infiltrate deep into eloquent brain areas<sup>(3)</sup>. For our analysis, we considered any effort of resection beyond GTR of CE as SPTR, irrespective of the procedure that was used to achieve this. While some studies had a relatively conservative SPTR procedure such as resection of additional residual 5-ALA fluorescent tissue<sup>(12,19)</sup> or resection of an additional rim ( $>1\text{cm}$ ) after GTR of CE<sup>(21)</sup>, Li et al. and Pessina et al. defined SPTR respectively as additional FLAIR abnormality resection (range  $>0\%$  to  $100\%$ )<sup>8</sup> and as  $100\%$  resection of T2-FLAIR hyperintense volumes in addition to GTR of CE<sup>(28)</sup>. Additionally, a very recent study defined SPTR differently as GTR plus (frontal or temporal) lobectomy, without any volumetric analysis on T2-FLAIR volumes or functionally defined borders during awake surgery<sup>(34)</sup>. Therefore, based on literature, SPTR can only broadly be defined as every effort of resection after GTR of CE portion of GBM; no standardized definition of SPTR exists and thus needs to be defined in a prospective setting.

Li et al. highlighted that in comparison with GTR, the relative low rates of post-operative new neurological deficits in the SPTR group could probably be explained by the increased use of awake surgery with neurophysiological monitoring, (sub)cortical mapping and imaging guidance such as intra-operative MRI, fMRI and DTI navigation.<sup>(2,8)</sup> Although Jenkinson et al. showed in a recent Cochrane review that intra-operative MRI or 5-ALA fluorescent guided surgery may help to increase the extent of GBM resection,

reports on adverse events were incomplete and studies had very low quality evidence.<sup>(2)</sup> Since portions of hyperintense T2-FLAIR area may represent both tumor infiltrated edematous and solely edematous portions, additional methods to delineate non-CE tumor infiltration are important to obtain a target for SPTR. Resection of the former may contribute to a better patient survival, while the impact of the latter on survival will be limited, but would pose an unnecessary risk of complications. In addition to 5-ALA, physiological pre-operative MRI imaging such as positron emission tomography (PET) MRI may help to identify tumor infiltrated, high metabolic portions outside the CE areas of GBM as a specific target during surgery.<sup>(35)</sup>

### Limitations

Overall, the results of our analysis should be interpreted with caution due to several limitations. First, because of between study heterogeneity ( $I^2=68\%$ ) across analyzed studies, which possibly arose due to several factors; the differences in defining SPTR; the heterogeneous or limited sizes of small study populations across studies; and their retrospective design and non-randomized treatment assignment. Indeed, as mentioned earlier, different definitions of SPTR across studies, which are often retrospectively defined and which currently varies between additional resection of fluorescing tumor to total resection of T2-FLAIR hyperintensity on top of GTR of CE, makes it challenging to draw a clear conclusion that would benefit clinical practice of neurosurgeons. Secondly, since the survival benefit and safety of resecting T2-FLAIR hyperintense regions is unclear, there was no clinical equipoise to randomize GBM patients into SPTR or GTR groups. Therefore, SPTR was probably performed in only a selected group of patients, since pushing the limits of GBM resection beyond CE was often not the main goal of surgery, especially not in tumors located in eloquent brain area.

Finally, a heterogeneous patient population with different prognostic factors such as age, KPS, adjuvant therapy, IDH mutation and MGMT methylation status, tumor volume and location were present across studies. Although corrections for some of these factors were applied in multivariate analyses across studies, the real impact of these factors on achieving SPTR and on survival are difficult to determine due to the retrospective nature of the studies. GBM location and eloquence is a known important prognostic factor. A very recent study suggests that GBM located in non-eloquent area such as the frontal or temporal pole could possibly receive safe SPTR with a total lobectomy.<sup>(34)</sup> However, no clear comparison with GBM located in eloquent area was available. In the articles we have included in our study, correction for GBM eloquence was performed only limited (as presented in Supplementary Materials). Furthermore, some of these factors such as genetic mutation, location and tumor size are inter correlated and their influence on survival is difficult to investigate retrospectively; GBM with IDH mutation (and MGMT methylation) have a significant better survival and at the same time are more often located in the frontal pole and have larger tumor size.<sup>(36)</sup>

These limitations introduced biases such as confounding by indication, information and selection biases in the analyzed studies, which consequently have influenced internal validity and the quality of the studies negatively. Indeed, since the quality of evidence of the analyzed studies were only moderate to very low, the benefit of SPTR of GBM on survival and its safety remains unclear and at this point of time and cannot be considered as a main goal of GBM surgery. Taking these limitations into account, we used a random effect model to provide a conservative, but clinically more reliable and meaningful interpretation of the treatment effect of SPTR<sup>(37)</sup>. The random effects model summary results of HR 0.47 (95% CI = 0.31 - 0.72) and median overall survival benefit of 6.4 months (95% CI % 3.2 - 9.7) provides a more reliable average estimate effect because it takes real differences in the SPTR versus GTR effect in each study into account and gives wider uncertainty around the estimate, as compared with fixed effects model summary results of HR 0.67 (95% CI = 0.58 - 0.78) and a median overall survival benefit of 4.6 months (95% CI = 3.9 - 6.0).

It should be underlined that SPTR should not be attempted at any cost. As always, the aim of GBM surgery remains to maximize the extent of GBM resection without causing new neurological deficits and by maintaining a good quality of patient's life. Any potential benefit of a prolonged survival by extending tumor resection could be considered by exploring (functional) boundaries during awake surgery and additional imaging adjuncts such intra-operative MRI, ultrasound and 5-ALA. This potential benefit should be weighed, together with the patient, against the risk of pushing the boundaries of GBM resection and its effect on post-operative neurological functioning and quality of life.

In our study, the incidence on post-operative new neurological deficits was not well reported across different studies. Post-operative neurological status was reported only descriptively, without the use of validated tests and quantitative outcome measures. Additionally, no data on quality of patient lives were reported across the studies. The safety of the procedure can therefore not be established.

## Future Directions

Future well designed, prospective, randomized controlled trials are thus needed to investigate both the safety and survival benefit of SPTR of GBM. These trials should include prospective, and clearly defined volumetric SPTR measures in order to standardize the definition of SPTR. Such a standardized definition of SPTR is expected to improve comparability between studies and improve the quality of evidence of studies investigating SPTR of GBM. Advanced physiological imaging techniques could additionally be used to identify non-CE infiltrating, residual tumor beyond the CE margins. Finally, validated measures on post-operative neurological outcome and quality of life assessment (QLQ-C30/QLQ-BN20) corrected for important prognostic factors such as age, KPS, adjuvant therapy, IDH mutation, and MGMT methylation status should be used to study the benefit of SPTR of GBM on patient survival and safety.<sup>(38)</sup>

## Conclusion

To the best of our knowledge, we performed the first meta-analysis on SPTR of GBM, suggesting that when compared with GTR, SPTR of GBM shows lower risk of mortality at any time during follow-up and longer overall median survival. These results should however be interpreted with caution, as the quality of the available evidence was only moderate to very low, and importantly, there was no consensus on the definition of SPTR and no reliable information on the safety of SPTR was available. Therefore, based on current available literature, the benefit of SPTR of GBM on survival and its safety remains unclear at this point of time and can as yet not be considered as standard of care for GBM surgery. Our findings however, does encourage further investigation in well-designed, prospective, randomized trials to clarify whether SPTR can be achieved safely to improve survival of patients with GBM.

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## Supplementary materials

### Search queries for several literature sources

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*('glioblastoma'/exp OR (glioblastom\* OR (maligna\* NEAR/3 glioma\*) OR (high\* NEAR/3 grade\* NEAR/3 glioma\*) OR ((grade-iv OR grade-4) NEAR/3 glioma\*) OR gbm):ab,ti,kw) AND ('surgery'/de OR 'neurosurgery'/exp OR 'cancer surgery'/exp OR 'brain surgery'/exp OR 'brain tumor'/exp/dm\_su OR 'surgical technique'/de OR (surg\* OR neurosurg\* OR resect\*):ab,ti,kw) AND ('extent of resection'/de OR 'surgical margin'/exp OR 'fluid attenuated inversion recovery'/de OR 'fluid attenuated inversion recovery imaging'/de OR 'gross total resection'/de OR (flair OR (Fluid NEAR/3 attenuat\* NEAR/3 invers\* NEAR/3 recover\*) OR t2 OR t-2 OR gross-total OR ((exten\* OR Supratotal\* OR Supramaxim\*) NEAR/3 (resect\* OR remov\*)) OR ((surg\* OR excis\* OR resect\*) NEAR/3 margin\*) OR ((beyond OR additional\*) NEAR/6 (contrast OR boundar\*))) :ab,ti,kw) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim)*

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#### Google Scholar

*glioblastoma|"malignant glioma"|"high grade glioma" flair|"Fluid attenuating inverse"|"gross total"|"Supratotal|Supramaximal resection"|"extent of resection"*

## GRADE quality ranking

Study	Quality level*	Underlying methodology	Risk of bias **	Precision
Aldave 2013 <sup>(12)</sup>	3	Non-random treatment allocation, retrospective	Moderate	Low N, wide CI
Li 2016 <sup>(8)</sup>	2	Non-random treatment allocation retrospective	Moderate	High N, narrow CI
Eyupoglu 2016 <sup>(19)</sup>	3	Non-random treatment allocation retrospective	Moderate	Moderate N, CI
Pessina 2017 <sup>(28)</sup>	3	Non-random treatment allocation retrospective	Moderate	Moderate N, CI
Esquanazi 2017 <sup>(18)</sup>	3	Non-random treatment allocation retrospective	Moderate	Low N, wide CI
Glenn 2018 <sup>(21)</sup>	4	Non-random treatment allocation, retrospective	Serious	Low N, wide CI

N = number of patients CI = Confidence Interval

\* Levels of quality (GRADE): 1: high, 2: moderate, 3: low, 4: very low

\*\* "Risk Of Bias In Non-randomised Studies - of Interventions" (ROBINS-I), based on risks of selection, information and confounding biases, with summarized judgements which includes moderate, serious and critical risks of bias or no information.

## Overview of multivariate analyses across studies with adjustment for prognostic factors.

10.1

Study	Multivariate analysis	Prognostic factors as variables
Aldave 2013 <sup>(12)</sup>	Yes	age, KPS, MGMT methylation status, tumor eloquent location, preoperative tumor volume, and adjuvant therapy.
Li 2016 <sup>(8)</sup>	Yes	unclear, at least treatment history
Eyupoglu 2016 <sup>(19)</sup>	No	Not performed
Pessina 2017 <sup>(28)</sup>	Yes	age, extent of contrast enhancement resection, tumor location
Esquanazi 2017 <sup>(18)</sup>	Yes	age, tumor volume, KPS, intra operative mapping, BCNU wafer
Glenn 2018 <sup>(21)</sup>	Yes	MGMT methylation status, tumor laterality

KPS = Karnofsky Performance Status, MGMT= methylguanine-DNA methyltransferase IDH= isocitrate dehydrogenase, BCNU= bis-chloroethylnitrosourea or Carmustine.



# Chapter 10.2

## Letter to the Editor: Supratotal resection of glioblastoma

Fatih Incekara, Marion Smits, Arnaud J.P.E. Vincent

*J Neurosurg.* 2019 Aug 16:1-2



## Supratotal resection of glioblastoma

TO THE EDITOR: We read with great interest the research by Roh et al. (Roh TH, Kang SG, Moon JH, et al: Survival benefit of lobectomy over gross-total resection without lobectomy in cases of glioblastoma in the noneloquent area: a retrospective study. *J Neurosurg* 2019 Mar 1:1-7. doi: 10.3171/2018.12.JNS182558. [Epub ahead of print]) regarding supratotal glioblastoma (GBM) resection in non-eloquent brain area.<sup>9</sup>

In this well written and timely article, the authors show that in patients with GBM located in right sided, frontal and temporal, non-eloquent brain area, supratotal resection (SupTR) defined as a total lobectomy improves median OS and PFS, when compared with solely gross total resection (GTR) of GBM. We commend the authors for critically appraising their clinical practice variations, which adds to our understanding on the potential value of SupTR of GBM in improving patient survival.

The study however, does raise some issues which need careful consideration. First, studying the value of SupTR without detailed and preferably standardized assessment of neurological outcome (i.e. NIHSS)<sup>10</sup> and quality of life assessments (i.e. QLQ-C30 and QLQ-BN20)<sup>11</sup> remains an important limitation. Although this issue is acknowledged by the authors, it is only briefly discussed in the limitation paragraph. Readers should be more strongly notified to not draw any decisive conclusions on the safety of this procedure even in noneloquent area, since only KPS was measured as a global neurological outcome and no data was available on the quality of patient life. Within this context, it also remains unclear how the dominant hemisphere and eloquent brain area were determined. The exclusion of all-left sided tumors by definition suggests that no functional MRI was used to assess functional lateralization and seems to disregard the notion that the right hemisphere may be dominant in some patients. Additionally, it should be noted that despite the exclusion of “tumors involving motor or language areas”, awake surgery was performed when GBM “was near an eloquent area”. We think that it would be beneficial to read in Table 1 how many cases were classified as (near) eloquent GBM and thus how many cases were performed with awake surgery. If this, hypothetically, was the case in the majority of patients, many noneloquent GBM should in fact have been labeled as (near) eloquent located GBM. In such a case, the conclusion that SupTR of noneloquent GBM is a safe procedure would - unintentionally - mislead the reader.

Secondly, as the authors acknowledge, the definition of supratotal resection is ambiguous. GBM is known to infiltrate beyond the margins of contrast enhancement, into the surrounding edematous T2/FLAIR-weighted hyperintense area, as seen on MRI.<sup>2</sup> In this study, SupTR was defined as complete resection of contrast enhancement as seen on T1-weighted postcontrast, postoperative MRI plus (frontal or temporal) lobectomy. In

addition, the authors considered a resection cavity on post-operative MRI that was larger than the contrast enhancing tumor volume on pre-operative MRI as a criterion for SupTR. We think that this criterion is highly debatable because of two reasons. First, factors such as brain shift and swelling of brain parenchyma makes a comparison of post-surgical cavity with pre-operative tumor volume unreliable. Secondly, as we mentioned earlier, GBM can infiltrate far beyond contrast enhancement and in fact, beyond the resection cavity as seen on post-contrast T1-weighted images, due to expansive and large T2/FLAIR-weighted hyperintense regions. The authors do not specify whether SupTR included the entire T2/FLAIR hyperintense region. In fact, unfortunately, no volumetric analysis on T2/FLAIR abnormality was presented in this study. Previously published studies investigating the role of supratotal resection or post-operative residual T2/FLAIR abnormalities found an association with survival.<sup>1,3-8</sup> We believe that currently no clear definition of supratotal resection exists and it can only broadly be defined as any effort of resection beyond the contrast enhancing part of GBM. In future studies it yet needs to be defined in a prospective setting.

Finally, we fear that the internal and external validity of the study is influenced by respectively confounding and selection biases. Although the authors acknowledge the presence of selection bias in the last sentence of the discussion, this issue needs more attention, since the impact of the biases on the results and the main conclusion is expected to be substantial. Indeed, out of 400 GBM patients only 40 patients with GTR of right-sided frontal or temporal noneloquent GBM were selected. It is also unclear how GBM location *within* a lobe and surgeon preference influenced SupTR vs GTR. The surgeon might have intended a SupTR for a GBM in the right temporal pole, whereas a more posterior and medial GBM within the same lobe might receive GTR instead of a lobectomy. Based on this highly selected group of patients, it remains unclear how to translate the potential benefit and the safety of SupTR for GBM surgery in general. In addition, the retrospective nature of the study prohibits a reliable comparison of median OS and PFS between the SupTR and GTR groups and raises several questions such as the impact of surgeon's preference on achieving SupTR vs GTR, its influence on survival and the impact of performing 5-ALA fluorescence guided or awake surgery on survival. It is therefore preferable to control for known confounding factors in a multivariate analysis and focus on the corrected hazard ratio of SupTR vs GTR, as the authors rightly performed, instead of a biased improvement of median OS and PFS between groups as seen in the Kaplan-Meier curves.

We hope that emphasizing these issues will encourage readers to carefully consider both the potential benefits *and* the risks of SupTR, such that the conclusions of this important study can be fully and reliably appreciated.



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# Chapter 11

## Summary & Conclusion



## Summary and conclusion

This thesis assessed the value of glioblastoma imaging and resection in light of molecular markers. Here, I summarize and discuss the main findings in this thesis. I provide future perspectives on topics addressed in this thesis and I finish with a conclusion.

In **Part I**, we predict molecular markers of glioma, primarily IDH mutation, 1p/19q codeletion and MGMT promoter methylation, and cognitive outcome of patients based on preoperative MRI scans. In **Part II**, we present the results of a randomized controlled trial that assesses the value of intraoperative ultrasound guided surgery on the extent of glioblastoma resection. We answer the question whether intraoperative ultrasound guided surgery enables complete tumor resection more often than standard glioblastoma surgery. Based on the results, we provide a recommendation to achieve maximal safe resection. In addition, we provide a proof of concept on the use of wearable mixed reality device for glioblastoma localization on the operating room. In **Part III** of this thesis, we study the association between the extent of contrast-enhancing and non-contrast enhancing tumor resection and survival of patients with glioblastoma. We validate our findings in a multi-center glioblastoma cohort. In addition, we assess the value of supratotal resection in glioblastoma, that is, tumor resection beyond the borders of contrast-enhancement.

## Main findings

### Part I: Preoperative imaging

In **Chapter 2**, we re-evaluated the topographical distribution of 436 glioblastoma with vs. without MGMT promoter methylation using voxel-wise MRI analysis in one of the largest homogenous IDH wildtype glioblastoma populations. Visual inspection indicated that when compared with MGMT unmethylated glioblastoma, methylated glioblastoma were more frequently located near bifrontal and left occipital periventricular area and less frequently near the right occipital periventricular area. These visual appearance of differences, however, could not be confirmed with rigorous voxel-wise statistical testing. We therefore conclude that there was no statistical difference in anatomical localization between IDH wildtype glioblastoma with vs. without MGMT promoter methylation. Prior to the WHO 2016 classification update,<sup>(1)</sup> studies on this topic had reported conflicting results; some studies showed that glioblastoma with MGMT methylation were lateralized to the left temporal lobe, while other showed right hemisphere lateralization.<sup>(2-5)</sup> There were also studies that reported no differences in localization, in concordance with the findings of our study.<sup>(6-9)</sup> These conflicting results in literature may partially be explained by variations in glioblastoma patient populations across studies, many of which were

performed in the pre-WHO 2016 classification era when the impact of molecular subtyping of glioblastoma according to IDH mutation status was less of a consideration.

In **Chapter 3**, we predicted the 1p/19q codeletion status in 284 low grade glioma patients on preoperative MRI scans with machine learning. We externally validated our algorithm on an independent dataset (n=129) and we compared the performance of our algorithm with that of clinical experts. We found that tumor location, heterogeneity on T2-weighted MRI scans, together with patient age and sex were the most important variables for our algorithm. We predicted the 1p/19q codeletion status of glioma with a performance that on average corresponded with experienced oncological neuroradiologists and outperformed oncological neurosurgeons. To the best of our knowledge, there are no prior studies predicting genetic mutations of low glioma on MRI using machine learning that compared their predictive performance with human performance.(10-13) Knowledge of the molecular profile of low grade glioma is clinically relevant, since it is recommended to consider them when timing postsurgical treatment with chemo- and/or radiotherapy. (14) Before clinical implementation in the future, more research is needed to improve the accuracy of AI algorithms, but we showed their potential to support decision making in clinical care of glioma.

In **Chapter 4**, we have developed and externally validated a deep learning algorithm that can accurately predict the IDH mutation status, 1p/19q co-deletion status, and grade of glioma, while simultaneously providing automatic glioma segmentation, based on pre-operative MRI scans. For patients in which tumor tissue cannot be obtained through surgical resection or diagnostic biopsy due to older age, poor neurological condition, or deep tumor localization, prediction of molecular markers on preoperative MRI scans may be clinically relevant in the future as an alternative.

In **Chapter 5**, we studied the association between the cognition of patients with a low grade glioma and white matter tract changes using diffusion tensor MRI and fiber tractography. We found that cognition in terms of language deficits in repetition of speech, imprinting and attention deficits were associated with changed microarchitecture of the arcuate fasciculus and the inferior fronto-occipital fasciculus. Our results support the hypothesis that glioma may infiltrate language white matter tracts and disrupt their microarchitecture, leading to deterioration of cognition in patients with glioma. Extensive glioma resection should be balanced carefully against persevering such infiltrated white matter tracts, preferably with awake surgery and stimulation mapping.(15)

## Part II: Image-guided glioblastoma surgery

In **Chapter 6**, we present the results of the ultrasound trial which aimed to assess the value of intraoperative ultrasound guided surgery on the extent of glioblastoma resection. The main research question was: *does intraoperative ultrasound guided surgery enable complete tumor resection more often than standard glioblastoma surgery?* We showed that intraoperative ultrasound enables complete tumor resection significantly more often (35%) than standard glioblastoma surgery (8%). We found that intraoperative ultrasound guidance did not harm patients in terms of neurological outcome, functional status and quality of life. Therefore, we recommended intraoperative ultrasound guided surgery, rather than standard surgery based on neuronavigation alone, to achieve complete safe tumor resection.

Complete resection of contrast-enhancing tumor has consistently been associated with longer overall survival in patients with glioblastoma.(16) Complete tumor resection is defined both qualitatively and quantitatively across studies.(17, 18) Some studies defined complete tumor resection as no residual contrast-enhancing tumor on a post-operative MRI scan, which is a stringent definition and may result in false positive assessment of residual tumor due to non-specific contrast enhancement such as due to ischemia, small vessels or unspecific reactive tissue enhancement.(19) To take interpretation variations into account, other studies also defined complete tumor resection as contrast-enhancing residual tumor  $<0.175 \text{ cm}^3$ (20, 21) or as a certain resection cut-off percentage (e.g.  $>98\%$ ). (22) It is known that residual tumor assessment of glioblastoma has a low interobserver agreement, introducing some degree of subjectivity when distinguishing contrast-enhancing residual tumor from non-specific contrast enhancement.(23) In our trial, we defined complete tumor resection as  $>99\%$  resection of contrast-enhancing tumor volume, accepting residual contrast-enhancing volume smaller than one percent to account for non-tumor related enhancement.

It is shown in two randomized controlled trials, that 5-aminolevulinic acid and intraoperative MRI guided surgery improve the extent of glioblastoma resection.(20, 21) However, an intraoperative MRI system is expensive and prolongs surgery time with approximately one hour.(21) This might explain why intraoperative MRI is still not implemented worldwide as a standard procedure during glioblastoma surgery. An alternative less-expensive, time effective technology that is used to acquire real-time imaging during brain tumor surgery is intraoperative ultrasound.(24) Real time imaging is needed, since neuronavigation systems are typically based on pre-operative MRI scans and due to brain shift, their accuracy in representing the actual situation during surgery decreases. As Jenkinson et al showed in a Cochrane review, no randomized controlled trial is ever performed to assess the value of intraoperative ultrasound to maximize tumor resection.(25) Therefore,

we initiated first such a trial and presented the results in this thesis. We recommend intraoperative ultrasound guidance as an alternative intraoperative imaging technology, just as intraoperative MRI or 5-aminolevulinic acid guided surgery (or a combination), to maximize the extent of contrast-enhancing tumor resection during glioblastoma surgery.

In **Chapter 7**, we tested in one of the first prospective clinical pilot studies, the clinical feasibility and accuracy of a wearable mixed reality device (Microsoft Hololens) for neurosurgical planning and glioblastoma localization on the operating room. We compared a wearable augmented reality headset with a standard neuronavigation system and showed in a proof of concept using quantitative outcome measures that augmented reality has a potential for glioblastoma visualization and localization during neurosurgical planning. We observed that manual hologram placement on the patient was a limitation decreasing the accuracy of tumor localization of the augmented reality device. For clinical implementation, the accuracy of the augmented reality device should be improved in future research, preferably through automated registration of the hologram with the patient and through integration of augmented reality with a neuronavigation system.

### **Part III: Extent of resection and survival**

In **Chapter 8**, we assessed the association between contrast-enhancing and non-contrast enhancing tumor resection and survival in light of MGMT promoter methylation in 326 patients with newly diagnosed IDH wildtype glioblastoma. This study showed an association between maximal contrast-enhancing tumor resection,  $\geq 30\%$  non-contrast enhancing tumor resection, minimal residual contrast-enhancing tumor volume and longer overall survival. Kaplan Meier estimates indicated that extensive surgery was potentially more beneficial for patients with MGMT methylated glioblastoma. Complete contrast enhancing glioblastoma resection is consistently associated with patient survival. (16) This thesis however, re-evaluates this association based on two new insights.

First, previous studies reported limited data on molecular characteristics of glioblastoma (i.e. IDH mutation and MGMT promotor methylation status) since many were performed prior to the WHO 2016 classification update, in which molecular sub classification was less of a consideration.(16) Although IDH mutation within newly diagnosed primary glioblastoma is rare ( $<5\%$ , and  $3.5\%$  in our cohort) these tumors represent a distinct molecular type of glioma arising from a distinct precursor lesion and the recent cIMPACT-NOW update suggests to call these tumors astrocytoma grade IV.(26-29) Therefore, new series are required to answer certain questions in tumor types defined according to this updated classification. We confirmed the association between complete contrast-enhancing tumor resection and longer survival in patients with glioblastoma IDH wildtype.



In **Chapter 9** we performed an international, multicenter, observational study including over one thousand patients with a newly diagnosed IDH-wildtype glioblastoma, in which we developed and externally validated a survival prediction model. This model included age, gender, preoperative Karnofsky performance status, extent of surgical resection, MGMT promoter methylation status, and adjuvant therapy and showed after external validation that survival of individual patients with a newly diagnosed glioblastoma could reliably be predicted. We published an online nomogram that could be useful to support shared decision making.

Secondly, this thesis re-evaluated the association between glioblastoma resection and survival by assessing the value of non-contrast enhancing tumor resection, beyond the borders of contrast enhancement. Glioblastoma is known to infiltrate far beyond the margins of contrast enhancement as seen on post-contrast T1-weighted MRI scans, into the surrounding edematous T2-weighted or T2-FLAIR hyperintense area.(30) This raises the question whether complete resection of contrast-enhancing tumor is indeed what is classically known as “gross total resection” or that resection should be extended beyond the margins of contrast enhancement, into the non-contrast enhancing area of signal abnormalities, to improve survival. The second question in this line, is which part of non-contrast enhancing tumor should be resected, as it can be hypothesized that only resection of tumor infiltrated, rather than edematous non-contrast enhanced tissue abnormalities will decrease tumor load and contribute to survival benefit. Physiological MRI is a promising tool to identify and distinguish tumor infiltrated portions from edema in non-contrast enhancing area.(31) We also showed in Chapter 8 that resection of  $\geq 30\%$  non-contrast enhancing tumor, beyond the margins of contrast enhancement, was associated with a longer overall survival. Other studies associated non contrast enhanced tumor resection percentages of 45%,(32) 53%(19) and 92%(33) with improved patient survival. Importantly, maximal glioblastoma resection should not be attempted at all cost and these studies reported very limited to no data on patient outcome. We also had insufficient data on patient outcome in our study, and therefore we recommended that intraoperative imaging and stimulation mapping should be used to pursue safe and maximal resection. In future research, the safety aspect of maximizing tumor resection needs to be further addressed.

The concept of supratotal resection has been known for some time within low grade glioma surgery, but it is only being investigated fairly recently in glioblastoma.(34) **Chapter 10.1** had the aim to study supratotal resection in glioblastoma surgery and its relation to patient survival in literature. We showed in our systematic review and meta-analysis based on six studies and 1168 glioblastoma patients that when compared with complete resection of contrast enhancement, supratotal resection of glioblastoma was associated with lower risk of mortality and longer median overall survival during follow



up. The quality of evidence however, was poor due to selection and confounding biases in the included studies.(19, 32, 35-38) In addition, there is much variation in the definition of supratotal resection for glioblastoma across studies. The safety aspect of supratotal resection was poorly reported, often lacking data on neurological outcome, complications or quality of patient life. Supratotal resection of glioblastoma can potentially provide survival benefit, however, future research is needed to assess its safety, as also addressed in our letter (**Chapter 10.2**).

## **Future perspectives**

It can be expected that in future neuro-oncological radiogenomics research, the accuracy of molecular and clinical outcome predictions on MRI will increase due to 1) the rise of artificial intelligence 2) more patient data as a result of large multi-center collaborations and consortia and 3) the addition of advanced MRI techniques including physiological MRI scans such as perfusion and diffusion and combination of MRI with PET-scanning.

Physiological MRI scans will not be only of benefit for radiogenomics research, but also for safely maximizing glioma resection due to a more accurate delineation of tumor margins than that we currently are able to do based on anatomical MRI scans only. (31) This may help us to understand which part of the non-contrast enhanced portion of glioblastoma is more tumor infiltrated and thus should be resected, and which part is more edema and can be preserved.

We further expect that new intraoperative imaging technologies, combined with artificial intelligence, will be of benefit to safely maximize the extent of brain tumor resection. Already, a recent study showed potential benefit of near real-time intraoperative detection of infiltration in tumor borders within minutes using stimulated Raman spectroscopy and deep neural networks to pursue maximal and safe glioma surgery, which may replace frozen sections during surgery in the future.(39) These technologies, when integrated in augmented reality navigation systems, may provide intraoperative guidance and decision-making during surgery, for example by overlaying accurate predictions of algorithms over tissue to determine whether that last part in the resection cavity is residual or healthy tumor tissue, which is often difficult to see even with a trained neurosurgical eye.

The future of neurosurgery will be shaped by new imaging technologies and artificial intelligence, and still with surgical experience, intuition and anatomical knowledge in its center, there will be a transition towards data driven surgery. It can be expected that this will not only contribute to maximize the extent of tumor resection, but the safety of brain tumor surgery will also be maintained by preserving functional brain area. Anatomical image guided surgery such as intraoperative MRI, intraoperative ultrasound and

fluorescent guided surgery will be supported by function-guided surgery such as awake surgery with stimulation mapping and intraoperative physiological image-guidance.

It can hopefully be expected, in short, that new imaging technologies and artificial intelligence will improve the way we diagnose and resect glioma, and thus eventually the way we provide clinical care for patients with brain tumors.

## Conclusion

This thesis contributes to our knowledge on the value of imaging and resection of glioma, in particular glioblastoma.

We presented promising results on molecular predictions based on preoperative MRI scans using artificial intelligence. We showed in a randomized controlled trial that intraoperative ultrasound enables complete tumor resection more often than standard glioblastoma surgery, without harming patients. We recommend intraoperative ultrasound guided surgery, rather than standard surgery, to achieve complete safe tumor resection. We found that augmented reality was potentially useful to visualize and locate glioblastoma for neurosurgical planning on the operating room.

Complete glioblastoma resection was already associated with improved patient survival. We confirmed this association in molecularly defined IDHwt glioblastoma. We externally validated these results in a multi-center glioblastoma cohort and predicted survival of patients with a glioblastoma with nomograms. In addition, we found that resection of non-contrast enhanced tumor beyond contrast enhancement or supratotal resection showed potential survival benefit for patients with glioblastoma. We encourage further research on the safety of maximizing glioblastoma resection, especially beyond the borders of contrast enhancement.

Finally, we shared future perspectives, in which we expect that new imaging technologies and artificial intelligence will improve how we understand, diagnose and surgically treat patients with brain tumors.

We hope that this thesis will be of benefit for future brain tumor research, clinical care and for patients with a brain tumor.

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## Samenvatting en conclusie

Dit proefschrift heeft de waarde onderzocht van glioblastoom beeldvorming en resectie in het licht van moleculaire markers. Hier geef ik een samenvatting en bespreek ik de belangrijkste bevindingen in dit proefschrift. Ik bied toekomstperspectieven op onderwerpen die in dit proefschrift aan de orde zijn gekomen en ik sluit af met een conclusie.

In **Deel I** voorspellen we moleculaire markers van gliomen, specifiek IDH-mutatie, 1p/19q codeletie en MGMT promotor methylering status, en cognitieve uitkomst van patiënten met een glioom op basis van preoperatieve MRI scans. In **Deel II** presenteren we de resultaten van een gerandomiseerde, gecontroleerde klinische studie die het nut van intraoperatieve echogeleide chirurgie op de uitgebreidheid van glioblastoom resectie onderzoekt. We beantwoorden de hoofdvraag of intraoperatieve echogeleide chirurgie vaker volledige tumorresectie mogelijk maakt, vergeleken met standaard glioblastoom chirurgie. Op basis van de resultaten geven we een aanbeveling hoe maximale veilige resectie te bereiken. Daarnaast laten we voor het eerst zien middels een concept idee dat augmented reality gebruikt kan worden in de operatiekamer om glioblastoom mee te lokaliseren. In **Deel III** van dit proefschrift onderzoeken we de associatie tussen de mate van resectie van contrast aankleurende en niet-contrast aankleurende tumor en overleving van patiënten met een glioblastoom. We valideren onze bevindingen in een multicenter glioblastoom cohort. Daarnaast onderzochten we het nut van supratotale resectie, dus tumor resectie buiten de grenzen van contrast aankleurings, bij glioblastomen op levensduur van patiënten.

## Hoofdbevindingen

### Deel I: Preoperatief imaging

In **Hoofdstuk 2** hebben we de topografische verdeling van 436 glioblastomen met vs. zonder MGMT promotor methylering geëvalueerd met behulp van voxel-gewijze MRI analyse in een van de grootste homogene IDH wildtype glioblastoom populaties tot nu toe. Visuele inspectie liet zien dat, vergeleken met MGMT niet-gemethyleerd glioblastomen, gemethyleerde glioblastomen vaker in de buurt van het bifrontale en linker occipitale periventriculaire gebied lagen en minder vaak in de buurt van het rechter occipitale periventriculaire gebied. Deze visuele verschillen konden echter niet worden bevestigd met voxel-gewijze statistische testen. We concluderen daarom dat er geen statistisch verschil was in anatomische lokalisatie tussen IDH wildtype glioblastomen met vs. zonder MGMT promotor methylering. Voorafgaand aan de update van de WHO 2016-classificatie (1) rapporteerden studies tegenstrijdige resultaten; sommige studies toonden dat

glioblastomen met MGMT methylering vaker in de linker temporale kwab zaten, terwijl andere lateraliserende epilepsie naar de rechterhersenhelft toonden. (2-5) Er waren ook studies die geen verschillen in lokalisatie rapporteerden, in overeenstemming met de bevindingen van onze studie. (6-9) Deze tegenstrijdige resultaten in de literatuur kunnen gedeeltelijk worden verklaard door variaties in de populaties van patiënten met glioblastoom, waarvan er vele werden uitgevoerd voorafgaand aan het tijdperk van de WHO van 2016 classificatie, toen de impact van moleculaire subtypering van glioblastomen op basis van IDH-mutatie status minder relevant geacht werd.

In **Hoofdstuk 3** voorspelden we de moleculaire marker, 1p/19q codeletie, in 284 laaggradige gliomen op basis van preoperatieve MRI-scans door gebruik te maken van machine learning. We hebben ons algoritme extern gevalideerd op een onafhankelijke dataset ( $n = 129$ ) en we vergeleken de prestatie van ons algoritme met die van klinische experts. We ontdekten dat tumor lokalisatie, tumor heterogeniteit op T2-gewogen MRI scans, samen met leeftijd en geslacht van de patiënt de belangrijkste variabelen waren voor ons algoritme. De nauwkeurigheid in het voorspellen van de 1p/19q codeletie status kwam gemiddeld overeen met die van ervaren oncologische neuroradiologen en presteerde zelfs beter dan oncologische neurochirurgen. Voor zover wij weten, zijn er geen eerdere studies die genetische mutaties van een laaggradige gliomen op MRI voorspellen met behulp van kunstmatige intelligentie die tevens hun algoritme vergeleken met artsen. (10-13) Kennis van het moleculaire profiel van laaggradige glioom is klinisch relevant, aangezien het aanbevolen is om hiermee rekening te houden bij het timen van behandeling met chemo- en of radiotherapie na een operatie. (14) Voordat deze bevindingen klinisch geïmplementeerd kunnen worden in de toekomst, is meer onderzoek nodig om de nauwkeurigheid van algoritmen te verbeteren, maar wij hebben hun potentiaal hier getoond om de besluitvorming in de klinische zorg voor gliomen te ondersteunen.

In **Hoofdstuk 4** hebben we een deep learning algoritme ontwikkeld en deze extern gevalideerd welke de IDH mutatie status, 1p/19q co-deletie status en glioom gradering nauwkeurig kan voorspellen en welke tegelijkertijd automatisch gliomen kan segmenteren, allemaal op basis van preoperatieve MRI scans. Voor patiënten bij wie tumorweefsel niet kan worden verkregen middels een operatie of diagnostische biopsie vanwege hogere leeftijd, slechte neurologische toestand of diepe tumor lokalisatie, kan een nauwkeurige voorspelling van moleculaire markers op preoperatieve MRI scans door algoritmen als alternatief klinisch relevant zijn in de toekomst.

In **Hoofdstuk 5** onderzochten we de associatie tussen cognitie van patiënten met een laaggradige glioom en veranderingen in zenuwbanen (witte stofbanen) met behulp van diffusie tensor MRI en tractografie. We ontdekten dat cognitie in de zin van taal

(herhalen van woorden), inprenting, en aandacht geassocieerd was met veranderde microarchitectuur van de fasciculus arcuata en de fasciculus fronto-occipitalis inferior. Onze resultaten ondersteunen de hypothese dat gliomen zenuwbanen verantwoordelijk voor taal kunnen infiltreren en hun microarchitectuur kunnen verstoren, wat leidt tot verslechtering van de cognitie bij patiënten met glioom. Uitgebreide glioomresectie moet zorgvuldig worden afgewogen tegen het intact laten van dergelijke geïnfilterde witte stofkanalen om functie te beschermen, bij voorkeur met een wakkere operatie en (sub)corticale stimulatie. (15)

## Deel II: Beeldgeleide glioblastoom operatie

In **Hoofdstuk 6** presenteren we de resultaten van de gerandomiseerde, gecontroleerde, klinische studie die het doel had om het nut van intraoperatieve echogeleide chirurgie te onderzoeken op de mate van resectie bij glioblastomen. De belangrijkste onderzoeksvraag was: maakt intraoperatieve echogeleide chirurgie vaker volledige tumor resectie mogelijk dan standaard glioblastoom chirurgie? We toonden aan dat met intraoperatieve echogeleiding significant vaker (35%) volledige tumorresectie bereikt werd dan met standaard glioblastoom chirurgie (8%). We zagen dat intraoperatieve echogeleiding patiënten niet schaadde in de zin van neurologische uitkomst, functionele status of kwaliteit van leven. Daarom bevelen we intraoperatieve echogeleide chirurgie, in plaats van standaardchirurgie, om volledige en veilige tumor resectie te bereiken.

Volledige resectie van contrast aankleurende tumor is vaker geassocieerd met langere overleving bij patiënten met een glioblastoom. (16) Volledige tumorresectie wordt zowel kwalitatief als kwantitatief gedefinieerd. (17, 18) Sommige studies definieerden volledige tumorresectie als 'geen contrast-aankleurende tumor rest' op een postoperatieve MRI scan, wat een strikte definitie is en kan leiden tot een fout-positief resultaat als gevolg van niet-specifieke contrast aankleuring, zoals ischemie, kleine bloedvaten of atypische aankleuring van reactief weefsel. (19) Om met dergelijke interpretatie verschillen rekening te houden, definieerden sommige studies ook volledige tumorresectie als 'contrast aankleurende tumor kleiner dan 0,175 cm<sup>3</sup> (20, 21) of als een bepaald resectie-afkappercentage (bijv. > 98%). (22) Het is bekend dat bij het beoordelen van tumor rest bij glioblastomen relatief veel interpretatie verschil zit tussen beoordelaars, wat een zekere mate van subjectiviteit introduceert bij het onderscheiden van contrast aankleurende tumorrest van aspecifieke contrast aankleuring. (23) In ons onderzoek hebben we volledige tumorresectie gedefinieerd als een resectie van > 99% van het contrast aankleurende tumorvolume, waarbij we een contrast aankleurende rest volume van minder dan één procent hebben geaccepteerd om met eventuele interpretatie verschillen rekening te houden.



In twee gerandomiseerde, gecontroleerde, klinische studies is aangetoond dat 5-aminolevulinezuur fluorescentie geleide chirurgie en intraoperatieve MRI geleide chirurgie de mate van glioblastoom resectie verhoogt. (20, 21) Een intra-operatief MRI-systeem is echter duur en verlengt de operatietijd met ongeveer een uur. (21) Dit zou kunnen verklaren waarom intraoperatieve MRI nog steeds niet wereldwijd wordt toegepast als standaard procedure tijdens glioblastoom chirurgie. Een alternatieve, goedkopere en tijdbesparende technologie die wordt gebruikt om terplekke beeldvorming te verkrijgen tijdens hersentumorchirurgie, is intraoperatieve echogelegeide chirurgie. (24) Real-time beeldvorming is noodzakelijk, aangezien neuronavigatiesystemen doorgaans gebruik maken van preoperatieve MRI scans. Echter, door verplaatsing van de hersenen tijdens het opereren neemt hun nauwkeurigheid bij het weergeven van de werkelijke situatie tijdens de operatie af. Zoals Jenkinson et al. liet zien in een Cochrane review, was er tot op heden geen gerandomiseerde, gecontroleerde, klinische studie uitgevoerd om de waarde van intraoperatieve echogeleiding te onderzoeken om glioblastoom resectie te maximaliseren. (25) Daarom hebben wij voor het eerst een dergelijk studie uitgevoerd en de resultaten hier in dit proefschrift gepresenteerd. We raden intraoperatieve echogeleiding aan als alternatieve intraoperatieve beeldgelegeide technologie, net als intraoperatieve MRI of 5-aminolevulinezuur fluorescentie geleide chirurgie (of een combinatie), om de mate van contrast aankleurende tumorresectie veilig te maximaliseren tijdens glioblastoom chirurgie.

In **Hoofdstuk 7** hebben we als één van de eerste prospectieve klinische pilotstudies, de klinische haalbaarheid en nauwkeurigheid van een dragbare augmented reality bril (Microsoft HoloLens) getest voor gebruik in de operatiekamer bij het plannen van glioblastoom operaties. We vergeleken de augmented reality bril met een standaard neuronavigatiesysteem en toonden in een proof of concept met kwantitatieve uitkomstmaten aan dat augmented reality potentieel gebruikt kan worden voor visualisatie en lokalisatie van glioblastomen in het kader van neurochirurgische planning. We vonden dat het handmatig plaatsen van een hologram op de patiënt een beperking was die de nauwkeurigheid van de augmented reality bril verminderde. Voor klinische implementatie moet de nauwkeurigheid van de augmented reality bril daarom nog worden verbeterd in toekomstig onderzoek, bij voorkeur door geautomatiseerde registratie van het hologram op de patiënt en eventueel door integratie van augmented reality met een neuronavigatiesysteem.

### **Deel III: Mate van resectie en levensduur**

In **Hoofdstuk 8** hebben we de associatie tussen contrast aankleurende en niet-contrast aankleurende tumor resectie en overlevingsduur onderzocht in het kader van MGMT promotor methylering bij 326 patiënten met een nieuw gediagnosticeerd IDH wildtype

glioblastoom. Dit onderzoek toonde een verband aan tussen complete contrast aankleurende tumor resectie,  $\geq 30\%$  niet-contrast aankleurende tumor resectie, minimaal contrast aankleurende tumorrest en langere overlevingsduur van patiënten. Kaplan Meier grafieken lieten zien dat uitgebreide chirurgie mogelijk gunstiger was voor patiënten met een MGMT gemethyleerd glioblastoom.

Volledige contrast aankleurende tumor resectie was voorafgaand aan dit proefschrift al vaker geassocieerd met langere levensduur van patiënten. (16) Dit proefschrift onderzocht deze associatie echter op basis van twee nieuwe perspectieven.

Ten eerste rapporteerden eerdere studies beperkte gegevens over de moleculaire classificatie van glioblastomen (op basis van IDH-mutatie en MGMT-promotor-methylerings status) aangezien er veel werden uitgevoerd voorafgaand aan de update van de WHO 2016 classificatie, waarbij moleculaire sub classificatie minder een relevant geacht werden. (16) Hoewel IDH-mutatie bij nieuw gediagnosticeerd primair glioblastoom zeldzaam is ( $<5\%$  en  $3,5\%$  in ons cohort), vertegenwoordigen deze tumoren een andere moleculaire glioomtype, afkomstig van een andere precursorlaesie. (26, 27) Daarom is onvolledig of ontbreken van moleculaire gegevens over IDH mutatie en MGMT methylerings status, of het mengen van moleculaire subtypes bij het onderzoeken van het effect van glioblastoom resectie op overleving, ongewenst. In dit proefschrift hebben we de associatie tussen complete contrast aankleurende tumorresectie en langere overleving bevestigd in patiënten met een moleculair gedefinieerd glioblastoom.

In **Hoofdstuk 9** hebben we een internationale, multicenter, observationele studie uitgevoerd met meer dan duizend patiënten met een nieuw gediagnosticeerd IDH-wildtype glioblastoom, waarin we een voorspellingsmodel hebben ontwikkeld en deze extern hebben gevalideerd. Dit model omvatte leeftijd, geslacht, preoperatieve Karnofsky performance status, mate van chirurgische resectie, MGMT-promotor methylatiestatus en adjuvante therapie en toonde na externe validatie dat de overleving van individuele patiënten met een nieuw gediagnosticeerd glioblastoom betrouwbaar kon worden voorspeld. We hebben een online nomogram gepubliceerd dat bruikbaar kan zijn om gedeelde besluitvorming te ondersteunen.

Ten tweede liet dit proefschrift zien dat glioblastoom resectie geassocieerd is met overlevingsduur, door het nut van niet-contrast aankleurende tumor resectie te laten zien, voorbij de grenzen van contrast aankleuring. Het is bekend dat glioblastoom ver voorbij de contrast aankleurende grenzen, in de omgevende oedemateuze gebieden, infiltreert, wat zichtbaar is als T2-gewogen of FLAIR gewogen hyper intense gebieden op MRI scans.(28) Dit roept de vraag op of complete resectie van contrast aankleurende tumor daadwerkelijk compleet is, of dat resectie voorbij de grenzen van contrast aankleuring, in

de niet-aankleurende delen, uitgebreid moet worden om overlevingsduur van patiënten te verbeteren. De tweede vraag in deze lijn is welk deel van de niet-aankleurende tumor dan geresecteerd zou moeten worden, aangezien verondersteld kan worden dat alleen resectie van tumor geïnfiltreerde weefsel, en niet het oedemateuze hersenweefsel, de hoeveelheid tumor zal verminderen, en dus zal bijdragen aan het overlevingsvoordeel. Fysiologische MRI is een veelbelovend techniek om tumor geïnfiltreerde hersenweefsel te onderscheiden van oedeem in niet contrast aankleurende gebieden.(29) In Hoofdstuk 8 toonden we ook aan dat dat  $\geq 30\%$  niet-aankleurende tumor resectie, voorbij de grenzen van contrast aankleuring, was geassocieerd met een betere overlevingsduur. Andere studies hebben gevonden dat niet-aankleurende tumor resectie percentages van 45%,(30) 53%(19) en 92% (31) was geassocieerd met betere levensduur voor patiënten. Belangrijk is dat maximale resectie van glioblastoom niet koste wat het kost nagestreefd moet worden en over het algemeen wordt in voorgaande studies slechts beperkt over patiënt uitkomsten gerapporteerd. Ook wij hadden onvoldoende gegevens over patiënten uitkomsten in ons onderzoek en daarom adviseren we intraoperatieve beeldvorming en (sub)corticale stimulatie om functionele gebieden te identificeren en daarmee veilig en compleet te opereren. In toekomstig onderzoek moet de veiligheid van uitgebreide glioblastoom resectie verder onderzocht worden.

Binnen de laaggradige glioom chirurgie is het concept van supratotale resectie al langer bekend, maar bij glioblastomen wordt dit nog pas vrij recent onderzocht.(32) **Hoofdstuk 10.1** had tot doel om supratotale resectie bij glioblastomen en de relatie tot levensduur van patiënten te onderzoeken in de literatuur. We lieten in onze systematisch reviewe en meta-analyse gebaseerd op zes studies met 1168 glioblastoom patiënten zien, dat vergeleken met totale resectie, supratotale resectie van glioblastomen geassocieerd was met lager risico op overlijden en langere mediane overleving gedurende follow up. De kwaliteit van het beschikbare bewijs was echter laag door selectiebias en confounding in de geïnccludeerde studies.(19, 30, 33-36) Daarbij was er veel variatie in hoe supratotale resectie bij glioblastomen werd gedefinieerd tussen de studies onderling. De veiligheid van supratotale resectie was slecht gerapporteerd, waarbij vaak gegevens misten over neurologische uitkomst, complicaties en kwaliteit van leven van patiënten. Supratotale resectie van glioblastomen kan potentieel bijdrage aan het verbeteren van prognose van patiënten met een glioblastoom, maar meer onderzoek is nodig in de toekomst om de veiligheid ervan te onderzoeken, zoals ook aangegeven in ons eerdere brief (**Hoofdstuk 10.2**).

## Toekomstperspectief

We verwachten dat in toekomstig neuro-oncologisch radiogenomics onderzoek de nauwkeurigheid van moleculaire en klinische uitkomst voorspellingen op basis van MRI scans zal toenemen als gevolg van 1) de opkomst van kunstmatige intelligentie 2) meer patiëntgegevens als gevolg van grote multicenter samenwerkingen en consortia en 3) de toevoeging van geavanceerde MRI technieken, waaronder fysiologische MRI scans zoals perfusie en diffusie MRI gecombineerd met PET scan. Fysiologische MRI scans zal niet alleen nuttig zijn voor radiogenomics onderzoek, maar ook voor het veilig maximaliseren van glijoom resectie door een nauwkeurigere detectie van de tumor grenzen, beter dan we momenteel kunnen op basis van alleen anatomische MRI-scans. (29) Dit kan ons helpen te begrijpen welk deel van het niet-contrast aankleurende deel verwijderd moet worden vanwege tumor infiltratie en welk deel gespaard kan blijven, omdat er alleen sprake is van oedeem.

We verwachten verder dat nieuwe intraoperatieve beeldvormende technologieën, gecombineerd met kunstmatige intelligentie, nuttig zullen zijn om de mate van tumor resectie veilig te maximaliseren. Een recent onderzoek toonde al het potentiële voordeel aan van intra-operatief beoordelen van tumor grenzen op infiltratie, waarbij binnen enkele minuten met behulp van gestimuleerde Raman-spectroscopie en kunstmatige intelligentie informatie werd verkregen om zo maximale en veilige glijoomchirurgie na te streven, die in de toekomst mogelijk vriescoupes zou kunnen vervangen. (37) Deze technologieën, eventueel geïntegreerd met augmented reality-navigatiesystemen, kunnen intraoperatieve navigatie en besluitvorming tijdens chirurgie ondersteunen, bijvoorbeeld door nauwkeurige voorspellingen van algoritmen over weefsel te projecteren om te beslissen of dat laatste deel in de resectieholte resterend tumor of gezond weefsel is, wat vaak moeilijk is te zien zelfs voor een geoefend neurochirurgisch oog. De toekomst van neurochirurgie in die zin zal worden gevormd door nieuwe beeldvormende technologieën en kunstmatige intelligentie, waarin een transitie zal plaatsvinden naar data gedreven chirurgie, waarin nog altijd chirurgische ervaring, intuïtie en anatomische kennis centraal zullen staan in besluitvorming.

Er kan verwacht worden dat dit niet alleen zal bijdragen aan het maximaliseren van de mate van tumor resectie, maar dat ook de veiligheid van hersentumorchirurgie ook zal worden verbeterd door behoud van functioneel hersengebieden. Anatomische beeldgeleide chirurgie zoals intra-operatieve MRI, intraoperatieve echografie en fluorescente geleide chirurgie zullen worden ondersteund door functiegeleide chirurgie zoals een wakkere operatie met elektrische (sub) corticale stimulatie en intraoperatieve fysiologische beeldgeleiding.

Kortom, er kan verwacht worden dat nieuwe beeldvormende technologieën en kunstmatige intelligentie de manier waarop we glioblastomen diagnosticeren, reseceren

en daarmee uiteindelijk de manier waarop we klinische zorg bieden aan patiënten met hersentumoren, zullen verbeteren.

## **Conclusie**

Dit proefschrift draagt bij aan de kennis over de waarde van beeldvorming en resectie van gliomen, in het bijzonder van glioblastomen.

We presenteerden veelbelovende resultaten over moleculaire voorspellingen van gliomen op basis van preoperatieve MRI scans met kunstmatige intelligentie. We toonden aan dat intraoperatieve echogeleide chirurgie vaker tot volledige tumor resectie resulteerde dan met standaard glioblastoom chirurgie, zonder daarbij patiënten te schaden in de zin van neurologische uitkomst, functionele status en kwaliteit van leven. We bevelen intraoperatieve echogeleide chirurgie aan in plaats van standaardchirurgie om een volledig veilige tumorresectie te bereiken. We hebben laten zien dat augmented reality potentieel nuttig was om glioblastomen op de operatiekamer te visualiseren en te lokaliseren in het kader van neurochirurgische planning.

Voorafgaand aan dit proefschrift, was volledige glioblastoom resectie al vaker geassocieerd met een betere prognose voor patiënten met een glioblastoom. We bevestigden deze associatie in moleculair gedefinieerde glioblastomen volgens de geupdate WHO 2016 classificatie. We hebben deze resultaten extern gevalideerd in een multicenter glioblastoom cohort en we voorspelden de overleving van patiënten met een glioblastoom met nomogrammen. Bovendien lieten we zien dat resectie van niet aankleurende tumor deel voorbij de grenzen van contrast aankleuring, dus supratotale resectie, potentieel bijdraagt aan een langere overleving bij patiënten met een glioblastoom. We moedigden verder onderzoek aan naar de veiligheid van het maximaliseren van glioblastoom resectie, vooral buiten de grenzen van contrast aankleuring.

Ten slotte deelden we toekomstperspectieven, waarin we verwachten dat nieuwe beeldvormende technologieën en kunstmatige intelligente zullen bijdragen aan onze kennis, diagnostiek en chirurgische behandeling van patiënten met een hersentumor.

We hopen dat dit proefschrift nuttig zal zijn voor toekomstig hersentumoronderzoek, klinische zorg en voor patiënten met een hersentumor.

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# Chapter 12

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PhD portfolio

About the author



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### **Adem Dereci**

Beste Adem, ik ben blij en vereerd dat je mijn paranimf bent. Je bent niet alleen mij studiegenoot, maar ook mijn familievriend waar ik altijd ogendicht op kan vertrouwen. Je bent overdag een clinicus in hart en nieren en in de avonden ben je altijd wel een case report aan het schrijven. Hoeveel heb je er nou nu?

### **Schoonfamilie**

Beste schoonfamilie, bedankt voor de interesse die jullie altijd in mijn onderzoek hebben getoond, voor het luisterend oor en voor al jullie steun tijdens mijn promotietraject.

### **Anne, baba, kardeşim Muhammed**

Bir ogul ve bir abi olarak istedigim kadar sizin ile vakit geciremedim. Kendimi bildim bileli ya kutuphanede yada hasthanede calisiyorum. Bunun ileridede cok degisecegini zannetmiyorum, bunu umitte etmiyorum. Anlayisiniz ve yardiminiz icin tesekkurler. Bu kitapçigi size armagan ediyorum.

Anne, baba, universitelerde tib olsun, felsefe olsun, bircok hocadan ders aldim. Hicbiri sizin bana verdiginiz Islami ve ahlaki degerlerden fazla ruhumda ve karakterimde etki birakmadi. Yaptigim ve ogrendigim herseyi bu degerler uzerine bina ettim. Bu yuzden bu kitapta, basarilarimda ve insallah basaracaklarimda sizin etkiniz cok buyuk. Ilime katki saglayabilme ve insanlara faydali olabilme umidi ile calismam sizi umarim mutlu eder. Sara yi buyutmede cok buyuk payiniz var, Merve ile bana cok destek oluyorsunuz. Allah sizden razi olsun. Sizi seviyorum.

### **Merve Ciftci-Incekara,**

Merveciğim, jouw steun en liefde speelt een grote rol in hoe ik mijn ambities in het leven hoopvol nastreef en realiseer. Je bent voor mij een onuitputtelijke bron van positieve energie en liefde. Ik ben dankbaar dat je mijn paranimf bent. Succes met je opleiding tot advocaat, jij kan het! Sara en ik zijn trots op jou en wij houden van jou.

**Sara Incekara**

Saracığım, sen annenin ve benim mutluluk kaynagimizsin. Umarım hayatın boyunca iman, ahlak ve ilim yolunda ilerlersin ve umarım bu kitapcık senin insanlığa faydalı bir hayat yasaman için bir motivasyon ve umit kaynağı olur. Seni seviyorum.

## PhD Portfolio

Name PhD Student: Fatih Incekara  
 Research school: Netherlands Institute for Health Sciences  
 Erasmus MC Departments: Radiology and Neurosurgery  
 PhD period: August 2016 - August 2020  
 Promotors: Prof. dr. Marion Smits and  
 Prof. dr. Martin van den Bent  
 Copromotor: dr. A.J.P.E. Vincent

PhD Training	Year	ECTS
<b>General courses</b>		
Clinical Epidemiology, Master of Science in Health Sciences (NIHES), courses:	2017-2019	70
Biostatistical Methods I: Basic Principles Biostatistical Methods II: Classical Regression Models Clinical Trials Clinical Epidemiology Fundamentals of Medical Decision-Making Principles of Research in Medicine and Epidemiology Clinical Translation of Epidemiology Principles in Causal Inference Methods of Public Health Research The Practice of Epidemiologic Analysis Conceptual Foundation of Epidemiologic Study Design	Logistic Regression Topics in Meta-analysis Advanced Topics in Clinical Trials Advanced Analysis of Prognosis Studies Advances in Clinical Epidemiology Causal Mediation Analysis Health Economics Markers and Prediction Research Biomedical English Writing Course Study Design	
Philosophy (of medicine), Bachelor of Arts (BA), courses:	2019-2020	90
Philosophy of Science I Philosophy of Science II Philosophy of Science III Descartes and Spinoza Thought Experiments The Early Enlightenment The High Enlightenment I: J.J. Rousseau The High Enlightenment II: I. Kant Aesthetics Thesis: Cartesian Consciousness, an interpretation beyond mind-body dualism	Biomedisch Ethiek Oefeningen in Perspectivisme Human Enhancement Moral Philosophy The Quest for Man I The Quest for Man II: S. Kierkegaard Eco philosophy Social and Political Philosophy Contemporary Challenges Critique	
Research integrity	2016	0.3
Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers (BROK)	2016	1.5
Basic course in applied MR techniques (ESMRMB)	2016	1
Diffusion MRI Course (ISMRM, Lisbon)	2016	1.5
Clinical fMRI & DTI Course (ESMRMB, London)	2014	1.5



<b>Conferences and presentations</b>	<b>Year</b>	<b>ECTS</b>
European Association of Neurosurgical Societies (EANS)	2020	1.5
Oral presentation: Intraoperative ultrasound guided surgery and the extent of glioblastoma resection: a randomized, controlled trial.		
Oral presentation: The association between the extent of glioblastoma resection and survival in light of MGMT promoter methylation in 326 patients with newly diagnosed IDH wildtype glioblastoma		
European Association of Neurosurgical Societies (EANS), Venice Dutch-Belgian Neurosurgical Societies, scientific meeting	2017	1.5
Posters (2x): Clinical feasibility and accuracy of a mixed reality device in neurosurgery		
Dutch Neurosurgical Society (NVvN), wintermeeting European Society for Neuroradiology, Rotterdam	2018-2019	1
Huisartsen Congres "Ontwikkelingen in de Geneeskunde", Rotterdam		
Oral presentations (3x): Clinical feasibility and accuracy of a mixed reality device in neurosurgery		
Dutch Neurosurgical Society (NVvN), wintermeeting	2019	0.3
Oral presentation: Artificial intelligence in oncological neurosurgery		
<b>Teaching activities</b>		
Supervising three medical students for master thesis	2017-2019	2
Fiber tractography and tumor segmentation in neurosurgery	2016	0.3
<b>Grants &amp; Awards</b>		
Best abstract award for oral presentation during EANS 2020: Intraoperative ultrasound guided surgery and the extent of glioblastoma resection: a randomized, controlled trial	2020	
Research grant awarded (40k) for the ultrasound trial, Stichting Coolsingel	2016	

## About the author

Fatih Incekara was born on February 14<sup>th</sup>, 1990 in Rotterdam. He studied medicine at the Erasmus Medical Center (2009-2015). As a second-year medical student, he started neurosurgical research in image guided neuro-oncological surgery, supervised by dr. Arnaud Vincent. In 2013, he spent 6 months as a visiting researcher at the department of neurosurgery, Brigham and Women's Hospital, Harvard Medical School in Boston, supervised by Prof. dr. Alexandra Golby. Fatih worked as a neurosurgical resident (ANIOS) at the Erasmus Medical Center for 9 months. During this time, he was awarded a research grant for the ultrasound trial, which eventually resulted in a part of this thesis.



In 2016, he started his PhD training on imaging and resection of glioma at the departments of Radiology and Neurosurgery, supervised by Prof. Marion Smits, Prof. Martin van den Bent and dr. Arnaud Vincent. During his PhD training, Fatih completed programmes in clinical epidemiology (Master of Science, NIHES) and philosophy (Bachelor of Arts, Erasmus University Rotterdam).

Fatih is married to Merve, together they have a daughter named Sara.

**my Lord, increase my knowledge**

Quran 20:114

**Medice, cura te ipsum**



